Alma Mater Studiorum – Università di Bologna

DOTTORATO DI RICERCA IN

SCIENZE VETERINARIE

Ciclo 33

Settore Concorsuale: 07/H2 – PATOLOGIA VETERINARIA E ISPEZIONE DEGLI ALIMENTI DI ORIGINE ANIMALE

Settore Scientifico Disciplinare: VET/04 – ISPEZIONE DEGLI ALIMENTI DI ORIGINE ANIMALE

INVESTIGATION ON EMERGING CONTAMINANTS IN FOODS OF ANIMAL ORIGIN

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Esame finale anno 2021

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1. INTRODUCTION

1.1 EMERGING CONTAMINANTS

1.1.1 What is meant by "emerging contaminants"

Over the past century, an uncontrolled development of a wide range of human activities has occurred globally, ranging from industry, transport, urbanization and agriculture. This strong increase in human activities has generated negative consequences for the environmental health at the level of air, water and soil. In the air there was mainly an increase in levels of CO2, particulate matter and other greenhouse gases. The level of water pollution has increased due to the use of a wide variety of chemicals, nutrients as well as oil and leachates spills. Finally, the soil was affected by the spread of pesticides, sludge, disposal of hazardous waste, use of dispo-sable goods or non-biodegradable materials, and lack of adequate waste facilities (Gavrilescu et al., 2015). All this has led to the spread in the environment of a series of contaminants.

Contaminants are defined as "substances (chemical elements and compounds) or groups of substances that that are toxic, persistent and liable to bioaccumulate" (Tornero et D'Alcalà, 2014).

For a long time now, however, is referring of this environmental contaminants with the adjective "*emerging"* (ECs). For many research groups (chemists, biologists, engineers, ecotoxicologists etc.) these represent one of the main topics of study. The interest in the presence of emerging contaminants can be traced back to the famous book published by Rachel Carson in 1962, Silent Spring (Carson R., 2002), which she showed how to use excessive Dichlorodiphenyltrichloroethane (commonly known as DDT) and pesticides had led to the death of many species, including those you play accompanied with their song the spring months (hence the title of the book). She was initially criticized because she seemed unwilling consider the benefits that the discoveries of science bring to humanity, but then the negative effects on health due to the use of DDT and some pesticides led to their ban and the writer's message in her book was re-evaluated and reconsidered (Sauvé et Desroisers, 2014).

We then continued to talk about emerging contaminants or pollutants, perhaps not always with the correct meaning. In fact, with "*emerging contaminants"*, attention is focused on compounds that are only recently in use or present. It would be better to use "*contaminants*

of emerging concern" (CECs) to underline that the attention is turned to new substances not yet monitored, or to substances that may also be already known, already in use and present in environmental compartments, but which only recently has the attention towards them because there have been changes in their use and disposal; or old compounds which, in the light of new scientific discoveries or experimental evidence, must again be the subject of research, investigations and monitoring to assess how much their presence in the different environmental compartments can be correlated to potential negative effects on human health and on environment more generally (Dulio et al., 2018).

Therefore, emerging contaminants are defined as a group of chemicals and their transformation products, of both synthetic and natural origin and / or any microorganism present that has the potential to enter the environment and cause known or suspected adverse effects on the environment and / or on human health, but which are not currently monitored in the environment (Geissen et al., 2015; Dey et al., 2019).

CECs and chemicals in general are ubiquitous, as they are found in water, air, domestic environments and food. There are growing concerns about the combo effects of this multitude of chemicals when they enter the environment and food chain. (Dulio et al., 2018), but for most of these compounds, environmental and human toxicology has not yet been studied (Paul et al., 2011), in particular regarding the definition of a Total Diet Intake (TDI) and Maximum Residue Level (MRL) in food. This making them a potential threat (Rosenfeld et al., 2011).

Their presence in the environment ranges from some μg/L to a few ng/L and sometimes even lower, so until recently it was impossible to discover, detect and monitor these persistent compounds at such low concentrations (Pal et al., 2014). Over the past decade, thanks to the development of new analysis techniques and complex detection methods, such as Liquid chromatography-mass spectrometry (LC-MS) and LC-MS / MS in particular (Geissen et al., 2015; Dey et al., 2019), which have a wide range of applications, detection of polar compounds such as most drugs, metabolites and transformation products that were previously not susceptible to analysis has been made possible (Kümmerer, 2011).

1.1.2 Categorization of emerging contaminants

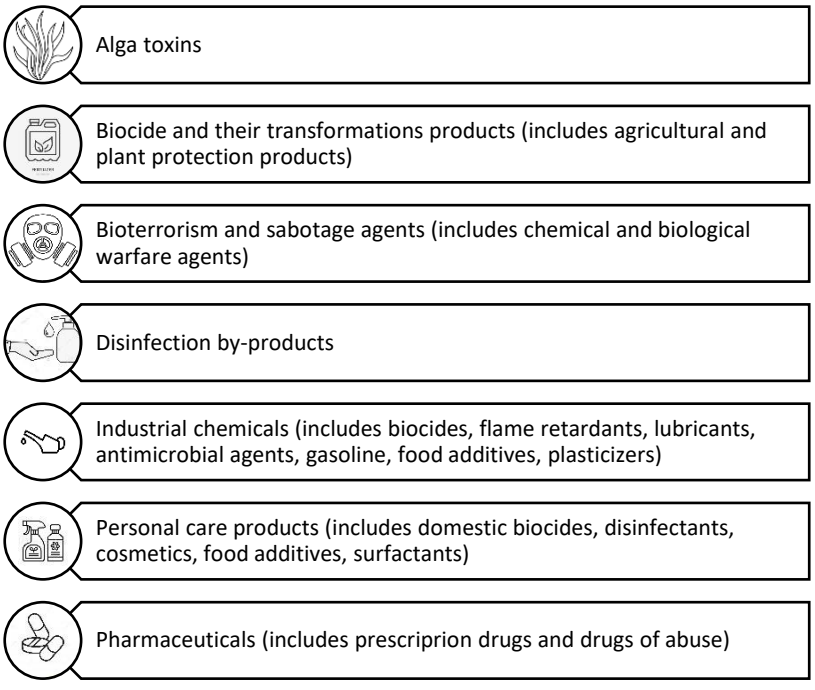
The range of compounds and chemicals that are part of this classification is very broad and with the introduction of new commercial chemicals, changes in the use and disposal of

currently widely used chemicals and further identification of new molecules continues to expand (Dey et al., 2019).

They are usually grouped together in classes according to their use, purpose or second some other feature. There is no standardization in their classification and some compounds can be included in groups different depending on the characteristics you want highlight and some groups therefore may overlap. The NORMAN project launched by the financial support of European Commission in 2005 aims to promote a permanent network of reference laboratories and research centers, integrating universities, industries, government regulatory agencies and non-governmental organizations (NORMAN, 2020). Over the past 10 years it has seen the participation of 70 members from more than 20 countries (Dulio et al., 2018). This project maintains one of the world's largest CECs classification records in the environment.

The NORMAN list can currently identify more than 1,036 CECs and their biotransformation products. Based on their origin and type, they are further divided into 30 categories (NORMAN Network, 2016). Figure 1.1 lists some of the main categories of ECs that are reported and cited in the literature. These include pesticides, drugs, pharmaceuticals and personal care products (PPCPs), disinfection by-products, endocrine disrupting compounds (EDCs), industrial chemicals, artificial sweeteners and food additives, sunscreens and UV filters, nanomaterials, flame retardants, benzotriazoles and benzothiazoles, siloxanes to name a few (NORMAN Network, 2016; Dey et al., 2019).

Figure 1.1. Classification and categorization of the main classes of emerging contaminants (Dey et al., 2019



Currently, international routine monitoring programs do not include any of the ECs (Geissen et al., 2015).

1.1.3 Persistent organic pollutants (POPs) and their dangerousness in food

Some ECs have physic-chemical characteristics that make them fall into the Persistent Organic Pollutants category. The term Persistent Organic Pollutants (POPs) refers to a particular group of carbon-based organic chemical contaminants that are persistent, bioaccumulative and have a long-range transport potential in the environment and pose a risk of causing adverse effects to human health (Guo et al., 2019). Some POPs, used for their pesticidal properties, were released into the environment as a result of an intentional process. Others, of industrial interest due to their properties, are emitted into the environment by volatilization, losses or accidental events during the entire life cycle of the product, from production to final disposal. Finally, others are formed in small quantities as by-products unwanted during high temperature processes and some industrial activities (Jones et Voogt., 1999).

Most POPs are halogenated chemical compounds. The strong bond that forms between the carbon atoms and the chlorine/bromine/fluorine atoms makes these pollutants very resistant to chemical, biological and photolytic degradation (Guo et al., 2019).

Therefore, once released, POPs can be transported to the atmosphere over long distances and remain in the environment for a long time. As a result of releases to the environment in recent decades due largely to human activities, POPs are now widely distributed across large regions (including those where POPs have never been used) and, in some cases, are found around the world, even reaching the arctic regions (Vorkamp et Rigét., 2014).

Some POPs have a half-life of years or decades and therefore remain for a long time in the environment, in this way it is easy for them to be absorbed by plants and animals (Guo et al., 2019).

POPs have a low water solubility and high lipophilicity, thus they are able to cross the phospholipid structures of biological membranes (Jones et Voogt., 1999) and bioaccumulate in living organisms. As they move along the trophic chain they can concentrate and biomagnify up to 70,000 times the initial values, reaching new toxicologically relevant levels. Particularly fish, predatory birds, mammals and humans absorb the highest concentrations, due to the fact that they are at the top of the food chain. Exposure to these pollutants (especially at high levels) can cause various health problems,

such as endocrine disorders, cardiovascular disease, cancer, diabetes, birth defects, and dysfunction of the immune and reproductive systems (Guo et al., 2019).

Many substances are toxic but few have them properties necessary to be classified like POP. POPs differ from other compounds in that they have particular values of some chemical-physical properties that determine four important characteristics: persistence, bioaccumulation, toxicity and mobility in the environment (Jones et Voogt., 1999).

The criteria used to classify a substance as POP are in fact:

• *Transport over long distances:*

- vapor pressure <1000 Pa
- half-life time in air > 2 days
- experimental evidence of presence in remote areas

• *Persistence:*

- half-life time in water> 2 months
- half-life time in soil / sediments > 6 months

• *Bioaccumulation:*

- bioaccumulation factor (BAF) > 5000
- bioconcentration factor (BCF) > 5000
- log Kow > 5

• *High toxicity.*

It is assumed that over 90% of human exposure to POPs occurs through the chronic intake of contaminated food. In particular foods of animal origin, first of all fish, are the main source due to the high lipid content (Liem et al., 2000).

Currently, the assessment of human health risks associated with dietary exposure of POPs is one of the most challenging challenges in food safety. To assess the risk, data on dietary exposure levels on the toxic effects of POPs are needed. However, for most POPs, information on toxicity is very scarce, partly due to the fact that for most POPs they have chronic and long-term effects. It is therefore difficult to evaluate and determine the dose-response relationship between POP and potential health problems (Guo et al., 2019).

With the aim of protecting consumers from POP-contaminated food, many national and international agencies such as the European Food Safety Authority (EFSA),World Health Organization (WHO), US Food and Drug Administration (FDA) and US Environmental Protection Agency (EPA) have developed regulations and guidelines to reduce the exposure to POPs (Guo et al., 2019).Among these, the most relevant is the Stockholm Convention, adopted on May 22, 2001 in Stockholm, Sweden and which entered into force on May 17, 2004. To date, more than 170 countries have ratified the Convention. The Convention requires interested parties to take measures to eliminate or limit the production and use of certain hazardous chemicals on the persistent organic pollutants list in the convention. The initial list included 12 POPs known as the "dirty dozen" (included Aldrin, Chlordane, DDT, Dieldrin, Endrin, Heptachlor, Hexachlorobenzene (HCB), Mirex, Toxaphene, Polychlorinated biphenyls (PCB), Polychlorinated dibenzo-p-dioxins (PCDD), Polychlorinated dibenzofurans (PCDF). Currently, up to Jul 2019, there are over 30 chemicals on the List of Persistent Organic Pollutants in the Convention. The list is divided into three annexes, with different degrees of restrictive measures:

- **Annex A (Elimination)**: the production and use of chemicals listed in Annex A should be eliminated unless there are specific exemptions;
- **Annex B (Restriction)**: the production and use of chemicals listed in Annex B must be limited;
- **Annex C (Unintentional Production)**: measures need to be taken to reduce the unintended releases of chemicals listed in Annex C.

The list of substances contained in the annexes is shown in Tables 1.1, 1.2 and 1.3.

Table 1.1. Chemicals listed in Annex A of the Stockholm Convention

Annex A	Aldrin
	Chlordane
	Chlordecone
	Dieldrin
	Endrin
	Mirex
	Heptachlor
	Hexabromobiphenyl
	Hexabromocyclododecane (HBCD)
	Hexabromodiphenyl ether and heptabromodiphenyl ether
	Hexachlorobenzene (HCB)

	Alpha hexachlorocyclohexane
	Beta hexachlorocyclohexane
	Lindane
	Pentachlorobenzene
	Polychlorinated biphenyls (PCB)
	Technical endosulfan and its related isomers
	Tetra bromodiphenyl ether and pentabromodiphenyl ether
	Toxaphene
	Chlorinated naphthalenes
	Hexachlorobutadiene
	Pentachlorophenol
	ecabromodiphenyl ether (commercial mixture, c-decaBDE)
	Short-chained chlorinated paraffins
	Dicofol
	Perfluorooctanoic acid (PFOA) its salts and PFOA-related compounds

Table 1.2. Chemicals listed in Annex B of the Stockholm Convention

Annex B	DDT
	Perfluorooctane sulfonic acid (PFOS), its salts and perfluorooctane sulfonyl fluoride

Table 1.3. Chemicals listed in Annex C of the Stockholm Convention

Annex C	Polychlorinated dibenzo-p-dioxins (PCDD) (“dioxins”)
	Polychlorinated dibenzofurans (PCDF) (“furans”)
	Hexachlorobenzene (HCB)
	Pentachlorobenzene
	Polychlorinated biphenyls (PCB)
	Polychlorinated naphthalenes (PCNs)
	Hexachlorobutadiene (HCBD)

Currently, the following chemicals are under review by the POP Review Committee to become part of the substances listed in the convention (POP Review Committee, 2019)

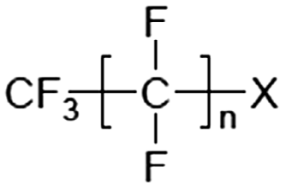
- Dechlorane Plus
- Metossiclor
- PV-328
- Perfluorohexane sulfonic acid (PFHxS), its salts and PFHxS-related compounds.

1.2 PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS)

1.2.1 Brief history and chemical properties

A family of emerging contaminants of recent concern is per- and poly-fluorinated alkyl substances (PFAS). PFAS are a class that includes thousands of substances of anthropogenic origin consists of a hydrophobic alkyl chain of varying length (usually C4–C16), in which one or more carbon-hydrogen bonds are substituted by carbon-fluorine bonds (following an electrochemical fluorination process) and a hydrophilic functional end-group (Chiesa et al., 2018). The hydrophobic portion can be linear, branched and partially or totally fluorinated, according to the formula "C_nF_{2n+1}"(Buck et al., 2011). The chemical structure of perfluoroalkyl substances, where "X" represents the terminal hydrophilic group, is shown in Figure 1.2

Figure 1.2. General structure of PFASs (EFSA, 2020)

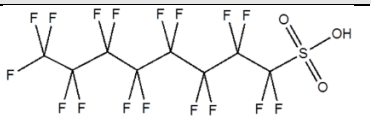
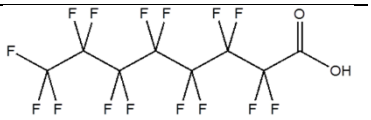
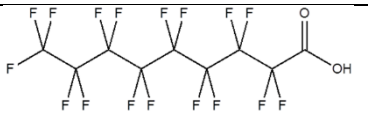
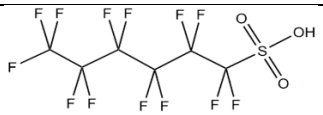


When all the hydrogen atoms are replaced by fluorine atoms, we speak of perfluorinated compounds (PFCs). The terminal hydrophilic group, instead, imparts to the molecule a neutral, positive or negative charge so that these compounds can be present in the form of cationic, anionic or neutral surfactants (ISPRA, 2019; EFSA, 2020). PFAS functional terminal groups include carboxylates, sulfates, sulfonates, phosphates, amines, and others. These functional groups, including dissociated and undissociated forms, govern many fate and transport properties of PFAS (ITRC, 2020).

The best known and most studied PFASs (See table 1.4) include Perfluorooctanoic acid (PFOA), Perfluorooctane sulfonate (PFOS), Perfluorohexane sulfonic acid (PFHxS) and

Perfluorononanoic acid (PFNA) to name a few. PFOA and PFNA have a have a final carboxyl group, instead PFOS and PFHxS have a polar sulfate group.

Table 1.4. Molecular formula, structural formula and molecular weight of some of the main PFAS (EFSA, 2020).

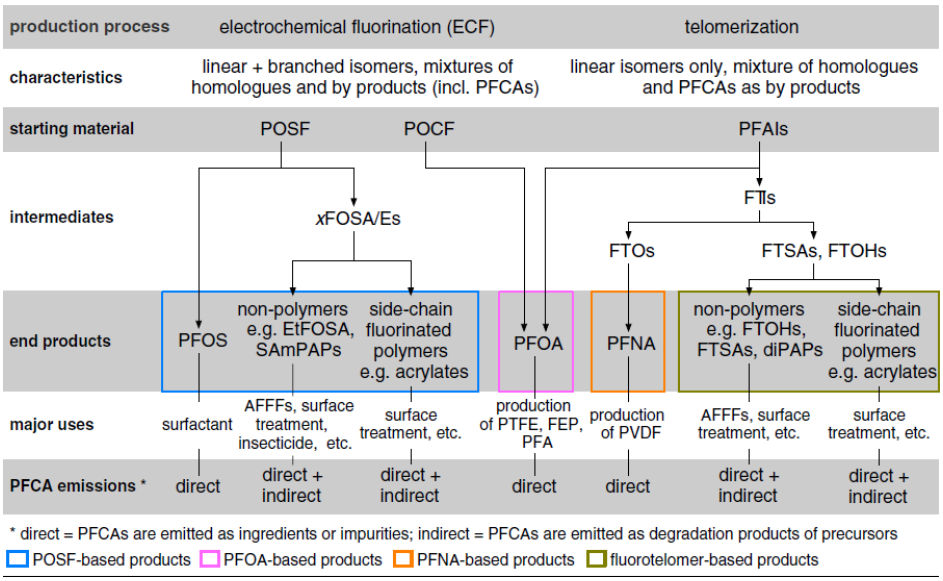
Compounds	Molecular formula	Molecular weight	Structural formula
PFOS	C8HF17O3S	538.22 (Potassium salt) 500.13 (Acid)	
PFOA	C8HF15O2	414.07	
PFNA	C9HF17O2	464.08	
PFHxS	C6HF13O3S	400.11/399.10	

They have been produced since the 1940s by 3M, by the main synthesis process named Electrochemical Fluorination (ECF). In this process, a linear hydrocarbon chain, subjected to an electric current, react with "HF" until the complete replacement of the "H" with atoms of "F". PFOS is the main product of this chemical reaction but not the only one, as the electrolysis of the hydrocarbon chain also leads to the formation of a mixture of compounds consisting of 4-9 carbon atoms (Lindstrom et al., 2011). This technology, providing for other low synthesis costs, was used until 2002, year in which 3M voluntarily stopped the production of PFOS also following taxes from the European Union, motivated by the presence of PFAS in the environment and in the industrial workers. The reduction in the use of PFOS has brought to light the significant emission of this compound, which has been gradually replaced by other chemicals, obtained through the telomerization process (Van Asselt et al., 2011). This second synthesis process consists of a reaction involving tetrafluoroethylene (a taxogen, CF2 = CF2) and pentafluoroethyl iodide (a telogen, CF3-CF2I) and leads to the formation of perfluorinated linear polymer chains and long chains

with even numbers of carbon atoms which can generally be split in two. The final reaction products, compared to those obtained by electrochemical fluorination, have the advantage of being pure, also due to the fact that the starting material is just as pure (EFSA, 2008). The sources of PFAS present in the environment can be divided into direct and indirect. Direct sources relate to production and use, while indirect sources are due to reaction impurities and transformations of (bio) degradation of compounds similar to PFAs with alcohols ozonolysis.

PFAs are used in a broad range of consumer products and industrial applications, in fact due to these chemical characteristics given by the strength of the bond between carbon and fluorine, they show an extraordinary thermal and chemical stability, resistance to photolysis, hydrolysis and metabolic and microbial degradation, as well as non-flammability and surface-active properties. Furthermore, the fluorinated alkyl chain is hydrophobic and lipophobic, immiscible, therefore both in water and in oil; this feature makes PFAS particularly useful as surfactants, capable of lowering the surface tension of water. The polymers of PFAS, whose fluorinated tails are exposed in the molecule, thus giving repellence to water and oil, have been widely used in oil-, water- and stain-resistant coatings for clothing, personal protective equipment and workwear as well as leather and carpets, oil-resistant coatings for food contact paper, aviation hydraulic fluids, fire-fighting foams, paints, adhesives, waxes, polishes, in industrial applications as surfactants, emulsifiers and coatings and personal care products including cosmetics (EFSA, 2020). Therefore they have a multitude of industrial applications, but currently information relating to the volumes of production for each of these uses is not publically available (EFSA, 2020). Figure 1.2 reported by Wang et al., 2014 shows general information on the production and uses of PFOS, PFOA and PFNA, as well as on their type of emission into the environment.

Figure 1.2. General information on the production and uses of PFOS, PFOA and PFNA, as well as on their type of emission into the environment (Wang et al., 2014)



On the other hand, their chemical-physical characteristics make these substances extremely persistent and bioaccumulative with potential adverse impacts on human health and environment (Su et al., 2017). Physico-chemical characteristics of the PFOS are listed in the table 1.5.

Table 1.5. Physico-chemical characteristics of PFOS (EFSA, 2008)

Physicochemical properties	***Value***
Appearance at ambient temperature and pressure	White powder
Melting point:	> 400 °C
Vapor pressure:	3.31 x 10-4 Pa at 20 °C (3.27 x 10-9 atm)
Air/water partition coefficient	$< 2x10^{-6}$
Solubility: pure water	570 mg/L
Henry's Law constant in water at 20 °C	4.34 x 10-7
pK_a	-3,3

1.2.2 Environmental pollution from PFAS.

PFAS can be released into the environment during production, use and disposal (EFSA, 2020). PFOS and PFOA are highly soluble in water and typically present in solution as anions, sometimes conjugated to bases, and have a very low volatility due to their ionic nature (ATSDR, 2009). Furthermore, PFAS have a very low vapor pressure and therefore diffusion in the aquatic environment is thought to be the main modality for their subsequent propagation throughout the surface of the globe. Municipal wastewater treatment plants and waste landfills are important direct sources of PFAS in aquatic ecosystems. At least one or more PFAS have been detected in 90% of all European rivers, and have also been detected in drinking water. (EFSA, 2020). Also atmospheric deposition is an important contributor to their environmental spread (EFSA, 2020). In fact, as many of these compounds are persistent and relatively soluble, they may be transported long distances in water and also in aerosols. In addition, their volatile precursors can be transported over long ranges of the atmosphere (EFSA, 2020). These compounds are not easily degradable by the main natural processes, in fact, they are thermally and chemically stable and resistant to biodegradation, atmospheric photo-oxidation, direct photolysis and hydrolysis. The chemical structure itself, in fact, is resistant to degradation: to break the carbon-fluorine bond it is necessary to provide a lot of energy and the fluorine atoms shield the carbonaceous skeleton of the molecule (EFSA, 2020).

PFAS have been found all over the world and in different matrices: in soil, aquifers (Hunter et al., 2019) surface water, rain (Sammut et al., 2017), polar caps (Maclnnis et al., 2017) air (Harrad et al., 2020) plants (Eun et al., 2020), as well wildlife animals and in human serum. PFAS in fact have been detected in biota of the entire food chain, starting with invertebrates up to large mammals. Their long-range transport potential was confirmed by the detected levels in blood of arctic mammals, ocean birds and other species which generally lives only in remote locations far from human settlement (Lindstrom et al., 2011). Interestingly, in fact, PFAS although not volatile compounds have also been found in the air, sediments and fauna of the Arctic region, despite being geographically separated from any anthropogenic sources (Lindstrom et al., 2011). The highest concentrations were found in the areas close to the discharges of the industries that still use perfluoroalkyl substances. It has been documented in both Japan and the US that water from springs located near such industries is contaminated with PFAS in concentrations ranging from 1 to 1000 ng/L.

Ocean water, on the other hand, has a lower concentration by several orders of magnitude, from 0.01 to 0.1 ng/L (Lindstrom et al., 2011).
The PFAS are mobile in the ground and are able to reach the underlying aquifers. However, it is not entirely clear how these are transported to areas far from industrial plants or products that contain them. Three hypotheses have been formulated about possible transport routes:

• Direct ocean transportation;

• Diffusion by marine aerosol, hypothesis supported by the evidence that surfactants accumulate on the surface of the water;

• Long distance atmospheric transport of volatile fluoropolymers rich in alcoholic groups which subsequently degrade into PFOS and PFOA (Wallington et al., 2006).

But a characteristics that makes them particularly dangerous is that PFAS, due to to their structural properties, can bioaccumulate as they move through food webs (Lindstrom et al., 2011).
Compounds with a perfluoroalkyl chain length (number of carbons with fluorine bonds) > 8 are generally more bioaccumulative than those with <7 (Lindstrom et al., 2011). Generally the octanol–water equilibrium coefficient is usually reflecting the bioaccumulation potential for fat soluble compounds, while for PFASs, the partitioning to serum proteins is likely to be the main mechanisms (EFSA, 2020).
Studies reported that PFOS concentration in the Arctic marine food web is positively correlated with trophic levels resulting in a trophic enlargement factor (TMF) of 3.1 (Houde et al., 2006). The same increase observations were reported in a Lake Ontario food web and in the food web of bottlenose dolphins and other species of marine mammals, indicating that the animals feed higher up the food chain they had higher PFAS concentrations (EFSA, 2020). According to studies reported in the literature, trophic enlargement factors are higher in aquatic food webs than in terrestrial ones (EFSA, 2020). However, also in humans (Olsen et al., 2007) and in other mammals (Houde et al., 2006) a high level of bioaccumulation of different PFAS has been demonstrated; this inevitably also affects farm animals which are then used for human nutrition (EFSA, 2020).

1.1.3 *Toxicity and sources of exposure for humans*

Due to the abundant use of PFAS for the production of different materials, it is not difficult to understand how humans are exposed to these chemicals.

In humans PFOS and PFOA are rapidly absorbed in the gastrointestinal tract and widely distributed in the body. However they are not metabolized but are excreted through urine and faeces. By binding to proteins and not being metabolized, they accumulate in the body especially in serum (bound to albumin), liver and to a lesser extent in the kidney. Half-life in human serum is estimated at approximately 5 years for PFOS and 2–4 years for PFOA (EFSA, 2018), and in general for long-chain PFASs can exceed 3 years (EFSA, 2020). The serum half-life times of PFOA, PFOS, PFNA and PFHxS for humans are shown in Table 1.6.

Table 1.6. Half-life in serum of main PFAS (EFSA, 2008)

PFAS	Half-life in serum
PFOA	3,8
PFOS	5,4
PFNA	3,2
PFHxS	8,5

Many studies underline relations between PFAS exposure and adverse immune outcomes in children, in particular PFOS gives antibody response at vaccination. Dyslipidemia, especially the increase in serum total cholesterol, is a critical effect associated with PFAS exposure (Sunderland et al., 2019; EFSA, 2018). Moreover, some studies reported a reduced birth weight and an increase in the serum levels of the liver enzyme alanine aminotransferase. Epidemiological data don’t provide sufficient data for PFOS and PFOA carcinogenicity in humans (EFSA, 2020). Some studies report evidence for carcinogenicity limited to occupationally exposed individuals with extremely high concentrations (Sunderland et al., 2019). PFOA has been included by IARC (International Agency Research on Cancer) in group 2B which included the substances "possibly carcinogenic to humans". This category is used for agents, mixtures and exposure circumstances for which there is limited evidence of carcinogenicity in humans and insufficient evidence of carcinogenicity in experimental animals. It can also be used when there is inadequate evidence of carcinogenicity in humans but there is sufficient evidence of carcinogenicity in experimental animals.

The extensive use of PFASs has led to widespread and worldwide environmental contamination; in the 1990s, when it was discovered that PFAS were present in human serum and worldwide, regulatory agencies were called upon to carry out research into the development, toxicity and effects of these substances on human health.

For this and other reasons in 2009 PFOS and its salts were included as persistent organic pollutant (POP) in Annex B of Stockholm Convention and their manufacturing and use have been restricted, while only recently in 2019, PFOA and its salts have been included as POP in Annex A and this implies measures to be taken to eliminate the production and use. Besides, the POPs Review Committee, completed the risk profile and risk management evaluation for (PFHxS), its salts and PFHxS-related compounds. The Committee, adopted a decision recommending that the Conference of the Parties consider listing PFHxS, its salts and PFHxS in Annex A to the Convention without specific exemptions (POPRC-15/1 decision,). The European Union Regulation EU 2019/1021 on persistent organic pollutant prohibits the manufacturing, placing on the market and use of PFOS and its salts. Commission Delegated Regulation EU 2020/784 amends Annex I to Regulation (EU) 2019/1021, adding PFOA, its salts and PFOA-related compounds to banned POP compounds.

The studies carried out showed that humans can be exposed to PFAS in the following ways:

- *Occupational exposure:*
  the levels of PFAS in the blood of industrial workers where these substances are produced or used are much higher than those of ordinary citizens of the same area; in this case, inhalation and dermal contact are the main routes of contamination (ATSDR, 2009);

- *Non-occupational exposure:*
  contamination of food, followed by air is probably the main route of exposure for those who do not work in contact with PFAS (Jain et al., 2017). The most common modes of exposure for this category of individuals including consumption of food and water contaminated, breastfeeding for newborns (Barbarossa et al., 2013) (breast milk is contaminated with PFAS from the mother's blood), Inhalation and ingestion of contaminated air and household dust containing PFAS, direct contact

with PFAS-treated materials, such as stain-resistant carpeting, mainly for children through hand-to-mouth transfer (EFSA, 2020).

Dietary intake is considered as one of the main pathways for human exposure to PFASs, as well as drinking water and airborne (Jian et al., 2017). Their environmental diffusion can contaminate soil and water used in food production. PFASs can enter the animal food chain via feed, water and soil ingestion by foraging farm animals contaminating the products like milk, eggs and meat (EFSA, 2020). Food can be also contaminated through food packaging, or processing equipment containing PFAS (EFSA 2020)

Numerous scientific studies have been carried out to assess the PFAS contamination in different food products. Data published underline that fish and seafood in general are the most contaminated food categories (EFSA, 2018; Vestergren et al., 2012; Hlouskova et al., 2013; Barbarossa et al., 2016; Jian et al., 2017, Chiesa et al., 2018). A recent review summarizes that PFAS concentration in food items show the following trend: fish and shellfish > eggs and meat products > milk products and beverages > vegetables (Jian et al., 2017). This trend is duo to PFAS high potential for bioaccumulation and biomagnification (Jian et al., 2017).

In 2020 EFSA published its latest scientific opinion (EFSA, 2020) on the risk to human health from the presence of per- and polyfluoroalkyl substances (PFAS) in food. The novelty of this new opinion is that the assessment was performed for the sum of four PFASs: PFOS, PFOA, PFNA and PFHxS, substances found currently to “contribute most to the levels observed in human serum”, with similar effects in animals and similar toxicokinetics.

In previous opinion from 2018 (EFSA, 2018) EFSA fixed separate tolerable weekly intake (TWI) of 13 ng/kg body weight per week for PFOS and 6 ng/kg body weight per week for PFOA. In the last years EFSA has developed a harmonized framework when evaluating the potential "combined effects" of chemical mixtures in food and feed (EFSA, 2019).

According to this approach the 2020 opinion sets a group tolerable weekly intake (TWI) limit of 4.4 ng/kg body weight for the mixture of the four PFASs. The EFSA CONTAM Panel concluded that parts of the European population exceed this TDI, which is of concern. According to the opinion the main contributing categories to the combined exposure to PFOS, PFOA, PFNA and PFHxS were ‘Fish meat’ and ‘Fruit and fruit products’ and ‘Eggs and egg products’ for all population groups.

1.2.2 Methods of analysis for PFASs

PFOS and PFOA are normally measured by multi-analytic methods, which typically include other PFAS. These methods don't always measure the same PFAS, and some methods measure more compounds than others. But even when only PFOS and PFOA are determined, it is important to know that these compounds can be distinguished from other PFAS, which can coelute and have common ionic fragments (EFSA, 2018). Advances in analytical technology over the past decade that have made it possible to measure PFAS in food and environmental samples at the levels typically found. in particular the rapid development and diffusion of Mass Spectrometry (MS) and the increased availability of analytical standards has led to significant advances in methodology for PFAS with the measurement of many more individual PFAS and improvements in the limits of detection (LOD) of up to three orders of magnitude (EFSA, 2018). This analytical performance improvements have increased the overall performance of the analyzes, thus also improving the comparison of data between the laboratories participating in interlaboratory comparative studies (EFSA, 2020). It is very important to pay close attention in terms of quality control when determining PFAS in biological and food samples. The most important control measures include the use of procedural blanks to verify laboratory contamination (PFAS being ubiquitous and used in many commonly used materials), estimates of matrix recovery and effects and regular participation in laboratory comparisons. It is also important to use appropriate isotope dilution standards. All these precautions are necessary to guarantee a high quality of the analytical data (EFSA, 2018).
Instrumental detection of PFAS in biological samples is usually performed in the same way as food samples (EFSA, 2018) and methods with the best sensitivity and quality control consist of extraction and cleaning of samples followed by determination by liquid chromatography (LC) coupled with quadrupole tandem mass spectrometry (LC-MS / MS) with electrospray ionization (ESI) (EFSA, 2018). LC–MS/MS is used in general to measure neutral PFASs and to screen for a large number of non-ionic and anionic fluorinated surfactants used in food contact materials. However, the sample preparation methods can differ substantially, based on the type of matrix. Sample preparation usually involves a combination of protein precipitation, ion pair extraction (IPE), liquid-liquid extraction (LLE) or solid-liquid extraction (SLE) methods. For foods with a high fat content the most commonly used extraction is SLE using medium polarity solvents such as acetonitrile or methanol (EFSA, 2018). Recently, methods that include SPE are also used.

If further cleaning is required (in order to remove interfering compounds which can bring ion suppression or ion enhancement which can occur when co-eluting compounds are present in the extract) graphitized carbon dispersed with glacial acetic acid or purification by filtration is commonly used (EFSA, 2018).

Specialist columns designed for this application may be required to ensure proper separation of compounds, as analytical columns used for standard analysis may not be suitable for quantifying branched chain molecules (EFSA, 2018).

Because PFSAs are not very volatile, they are not amenable to direct gas chromatography (GC) analysis, and liquid chromatography (LC) analysis with ultraviolet detection is also not suitable as there is no suitable chromophore (EFSA, 2018). However, it is possible to use the GC if the PFAS first undergo a derivatization treatment in order to be converted into methyl-esters (more volatile). In this case, detection is possible using the electron capture detector or mass spectrometry (MS) (EFSA, 2018). Due to the better separation achievable with GC methods, derivatization followed by GC-MS is still sometimes used when greater resolving power is needed to separate coeluting isomers when using LC methods, such as for neutral and volatile PFAS (EFSA, 2018).

1.3 DECHLORANE PLUS AND RELATED COMPOUNDS (DRCs)

1.3.1 Brief history and chemical properties

Dechlorane, also known as Mirex, was widely marketed as a pesticide as well as a flame retardant (FR) in the USA from the 1960s to the 1970s (Brasseur et al., 2014). FRs are a wide range of chemicals generally used in the manufacture of electronic, textiles, plastics and building materials in order to inhibit the development and propagation of flames and therefore increase the level of safety of these products (Stapleton et al., 2014). There are different groups of FRs based on their chemical characteristics. One of these is represented by halogen-containing compounds which includes Dechlorane Related Compounds (DRCs). Due to its toxicity, persistence and high bioaccumulation potential, Mirex was banned in the United States in 1978 (Kaiser, 1978), consequently, other related compounds such as Dechlorane Plus (DDC-CO or DP), Dechlorane 601 (DDC-ID or Dec-601), Dechlorane 602 (DDC-DBF or Dec-602), Dechlorane 603 (DDC-Ant or Dec-603) and Chlordene Plus (DDC-PDD or CP), patented by former Hooker Chemicals and Plastics Corp. (Hooker; currently OxyChem, Niagara Falls, New York), were developed for the purpose of replacing Mirex (Brasseur et al., 2014). While DDC-CO, DDC-ID, DDC-DBF,

DDC-Ant, and DDC-PDD are the official abbreviations established by the scientific community in 2012 (Bergman et al., 2012), these compounds are more commonly referred to as respectively DP, Dec-601, Dec-602, Dec-603 and CP.

DP has a chemical formula of $C_{18}H_{12}Cl_{12}$ and a molecular weight of 653.70 g/mol (Xian et al., 2011). Commercially available formulation of DP (CAS No. 13560-89-9) contains two stereoisomers, syn-DP (CAS No. 135821-03-3) and anti-DP (CAS No. 135821-74-8), in the approximate ratio of 1:3 so the anti isomer represents 75% of the total (Sverko et al., 2011). All these compounds (see Table 1.7) are highly chlorinated and share a bicyclo [2,2,1] heptene structure, resulting from a Diels–Alder reaction between one or two hexachlorocyclopentadiene molecules and various cyclic dienophiles, as shown in figure 1.3. (Wang et al., 2016).

Figure 1.3 Diels–Alder condensation of hexachlorocyclopentadiene and 1,5-cyclooctadiene (Wang et al., 2016)

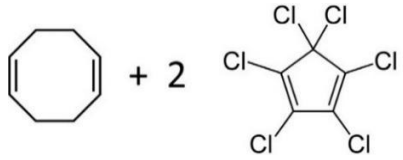
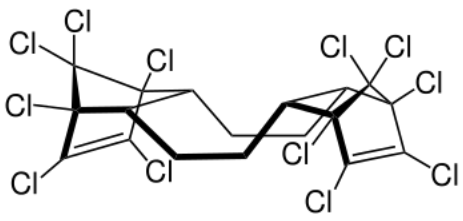
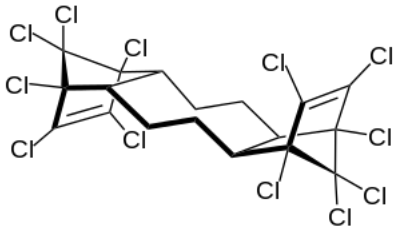


Figure 1.4. Structure of syn-DP and anti-DP (Wang et al., 2016)

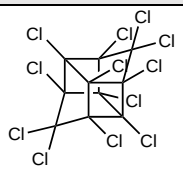
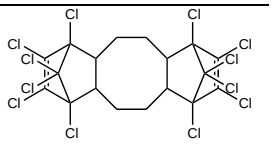
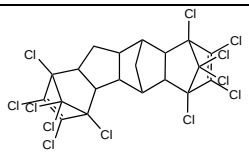
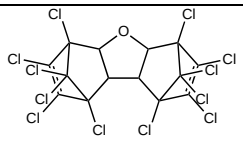
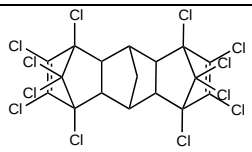
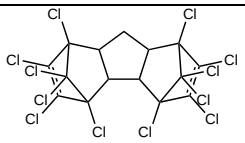


Syn isomer



Anti isomer

Table 1.7. Molecular formula and structural formula of Mirex and DRCs.

Compounds	Molecular formula	Structural formula
Mirex	$C_{10}Cl_{12}$	
Dechlorane Plus	$C_{18}H_{12}Cl_{12}$	
Dechlorane 601	$C_{20}H_{12}Cl_{12}$	
Dechlorane 602	$C_{14}H_4Cl_{12}O$	
Dechlorane 603	$C_{17}H_8Cl_{12}$	
Chlordene Plus	$C_{15}H_6Cl_{12}$	

DP has a crystalline, white and odourless appearance. Its physico-chemical properties are shown in Table 1.8. It reaches the melting point with decomposition at 350 °C and has a very low vapour pressure of 0.006 mm of Hg at 200 °C (Oxychem, 2011).

It is considered insoluble in water and is extremely lipophilic, having an octanol-water partition coefficient very high (Log Kow=9.3), indeed it has similar characteristics of other POPs, including resistance to biodegradation, and bioaccumulation (log BAF ~5 in fish) (Sverko et al., 2011).

Table 1.8. Physico-chemical properties of DP (OxyChem, 2011).

Property	Value
Molecular mass	653.7 u
Melting point	350 °C with decomposition
Vapor pressure (at 200°C)	0.006 mm of Hg
Water solubility	44 ng/L – 249 μg/L
Octane–water partition coefficient (Log K*ow*)	9.3

Since there are no natural sources of DP, its presence in the environment is given exclusively by anthropogenic sources. As a flame retardant, in fact, DP is used in many polymeric systems, in general thermoplastic or thermosetting. Examples of commonly used thermoplastics that may contain DP include nylon, polyester, acrylonitrile butadiene styrene, natural rubber, polybutylene terephthalate (PBT) and polypropylene and styrene butadiene rubber (SBR) (ECHA, 2020). DP is classified as a low production volume chemical in the EU, while it is categorized as a high production volume chemical in the USA. Only two manufacturers in the world synthesize these compounds: OxyChem (Niagara Falls, USA) with a current annual production of 450-4500 tons, and Anpo Electrochemical Co. (Jiangsu, China) with a volume production of 300-1000 tons/year (Wang et al., 2016). The global annual production is approximately 5000 tonnes (Ren et al., 2008).

1.3.2 General environment contamination and bioaccumulation

DP and related compounds are persistent in the environment, subject to long-range atmospheric transport, biomagnification and bioaccumulation in biota through the food chain. DP's half-life in water is estimated to more than 24 years, with minimal or no anaerobic degradation (Sverko et al., 2011), while in the soil Cheng et al. (2019) reported that after 260 days only 4.2-8.2% of the initial PD are degraded.

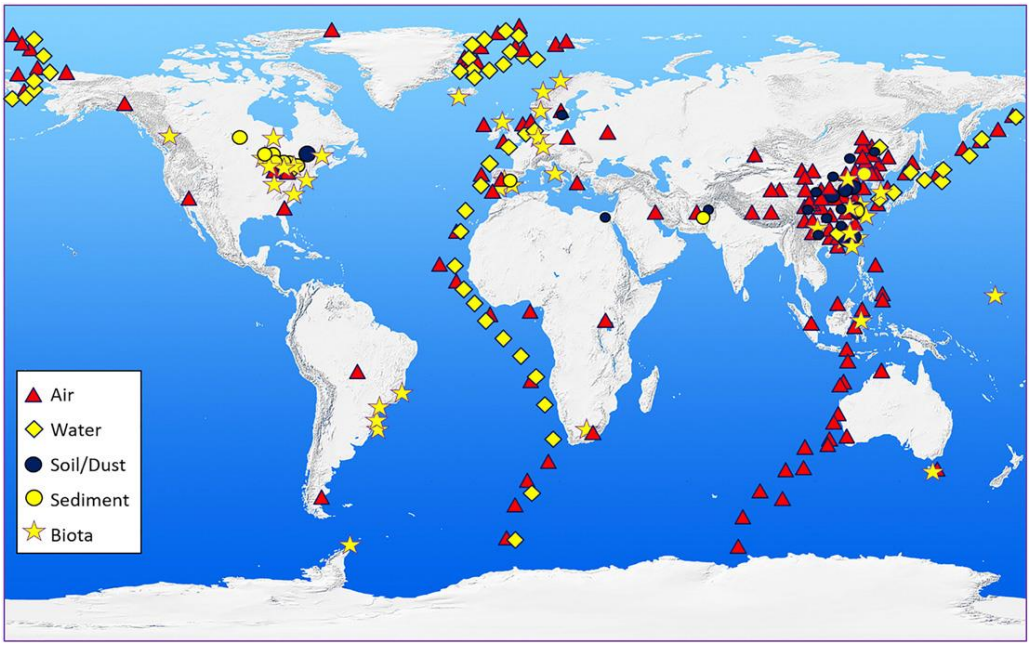
DP has been observed worldwide in the air and in the marine environment suggest that transport may occur both via air and seawater. Möller et al. (2010) were the first to show the potential for DP to undergo long-range environmental transport, as indicated by the detection of DP in remote ocean areas of the Arctic and Antarctica. In this study, marine

boundary layer air and surface seawater samples were collected during a sea expedition from the East-Greenland Sea, and in the Northern and Southern Atlantics toward Antarctica. DP concentrations ranged from 0.05 to 4.2 pg/m3 in the atmosphere and from not detected to 1.3 pg/L in seawater (ECHA, 2020). Soils and lichens from arctic regions were also found to be contaminated with DP, according to Gao et al. (2018). While only two production sites are known in the world, DRCs are ubiquitous substances worldwide due the chemical characteristics listed above and were reported in a wide range of different matrices. The first detection of DP was reported in 2006 in the Great Lakes Basin in North America (Hoh et al., 2006) in air, fish and sediments samples. Following this, other studies have been carried out over the years that highlighted the presence of DP in environmental matrices like air, water, soil and sediment (Moller et al., 2010; Tomy et al., 2007; Sverko et al., 2008; De la Torre et al., 2010; Wu et al., 2010; Chen et al., 2011; Klosterhaus et al., 2012; Gao et al., 2018)

The highest concentrations of DP was reported in ambient air (up to several ngm−3) and in soil (μg/g dw) close to the DP manufacturing facility in Huai'an City (Wang et al., 2010) and in sediment (μg/g dw) from an e-waste recycling site in South China (Zhang et al., 2011), indicating how the levels of DP reported in the environment are higher in the regions of origin near production sites and urban centers. In fact, in remote regions and oceans the levels are generally much lower, although in some cases high levels have also been observed in remote sites (Sverko et al., 2011). Many studies conducted in different parts of the world also confirmed the presence of DP in wild animals such as various species of fish by Tomy et al. (2007), Wu et al. (2010) Klosterhaus et al. (2012), Kang et al. (2010), Sühring et al. (2013 and 2016), Rjabova et al. (2016), Abdel Malak et al. (2018) Zacs et al. (2018). DP was detected also in birds (Klosterhaus et al., 2012; Gauthier et al., 2009; Guerra et al., 2011) and mammals (Klosterhaus et al., 2012; De la Torre et al., 2012; Vetter et al., 2015) demonstrating how it can accumulate in the most complex organisms through the food chain. This was also experimentally confirmed by a recent laboratory-scale microcosm fish study, that reported bioconcentration factor (BCF) values exceeding the bioconcentration criteria set by the Stockholm Convention (BCF >5000) (Wang et al., 2019; ECHA, 2020), confirms also in this case the biomagnification potential of DP.

In figure 1.5 reported from the work of Wang et al. (2016) all the geographical points worldwide where DP levels in the matrix such as air, water, soil/dust, sediment and biota have been detected are summarized. This providing a very clear overview of the ubiquity and widespread of these substances.

Figure 1.5 Global distribution of DP sampling sites (Wang et al., 2016)



DP was detected also in human samples, like hair (Qiao et al., 2018), breast milk (Siddique et al., 2012; Ben et al., 2013), blood (Ben et al., 2013), adipose tissue (Yin et al. 2020) and serum. Mean levels of DP observed in human serum from Canada, France, Korea and Norway compared to occupationally exposed people in China had median values of 2.39, 1.20, 0.73, 1.3 and 42.6 ng/g lw, respectively (Zhou et al., 2014; Brasseur et al., 2014; Kim et al., 2016; Cequier et al., 2015; Ren et al., 2009), demonstrating how people living near production and/or disposal of PD are more exposed to these substances. These data highlights that the contamination of the DRC is widespread geographically and biota, also affecting human's population.

1.3.3. Toxicity and sources of exposure for humans

Data regarding the toxicity of DRCs on human health are currently very limited, but some toxicology works present in literature have shown that oral exposure to DP can lead oxidative damage to the liver and alterations in metabolism and signal transduction in mice males (Wu et al., 2012). Other studies conducted by Barón et al. (2016) and more recently by Chen et al. (2019) on marine organisms demonstrated the genotoxicity of DP for mussels and showed that exposure to DP causes neurobehavioral abnormalities in zebrafish.

Toxicity studies in experimental animals suggest low concern for acute toxicity via the oral and inhalation routes of exposure. However, there are some data gaps, for example, there

are no long-term studies exceeding 90 days, which might be important given the apparently slow uptake of the substance. Therefore, toxicity testing has been required by ECHA (ECHA, 2020). As a result, there are currently insufficient elements to define human Tolerable Daily Intake (TDI) for DRCs and much less MRLs have been defined in food. Studies on the presence of DRCs in foodstuff matrices are also important for determining the level of exposure of the population, providing adequate data for the risk assessment. This studies, although limited and preliminary, provide concerns about the potential threat of PD to human health and ecosystems and makes it a compound of public safety concerns. For this reason DP has been classified into the Candidate List of Substances of Very High Concern by ECHA (ECHA, 2018), and is actually under review to become part of the substances listed in the Stockholm Convention (POPs Review Committee, 2019) having been determined that DP isomers meet the screening criteria specified in Annex D. DP has in fact all the characteristics necessary to be considered a POP, such as potential for long range environmental transport, persistence, bioaccumulation properties and ability to cause adverse effects, as summarized in Table 1.9.

Table 1.9. POP characteristics of DP and its syn- and anti-isomer (ECHA, 2020).

Criterion	Criterion satisfied (☑ or ☒)	Remarks
Persistence	☑	-Modelling of degradation potential and microbial metabolic pathways which suggests that biodegradation is slow, and low probability that DP will degrade any faster than structural analogues that are listed under the Stockholm Convention (ECHA, 2017) -DP half-life in water are more then 24 years (Sverko et al., 2011) -Limited degradation in ageing soil with only 4.2-8.2% of initial DP having degraded after 260 days (Cheng et al., 2019) -Lack of degradation in soil over time (Wang et al., 2010) and low ability to biotransform in fish (Tomy et al., 2008) support the conclusion that DP is persistent.

Bioaccumulation	☑	Log Kow of 9.3 (OxyChem, 2011) indicating high potential for bioaccumulation. -Depuration half-life, which is corresponding to a BAF >5000 (Wang et al., 2019)
Potential for Long-Range Environmental Transport	☑	DP is detected in the environment and biota in remote areas from the Arctic to Antarctic (Möller et al.2010; Gao et al. 2018).
Adverse effects	☑	Neurotoxicity as observed in zebrafish (Chen et al., 2019) and oxidative stress as observed in marine bivalves (Barón et al., 2016), and mice (Wu et al., 2012).

The human organism can absorb DRCs through inhalation of air (Hou et al., 2018) and ingestion of dust (Sun et al., 2018). Studies in the literature have shown that e-waste dismantlig workers and the populations living in areas near a waste recycling plantsin are more exposed to this type of absorption (Ren et al., 2009; Zheng et al., 2010). Perinatal exposure via blood and breast milk is also possible (ECHA, 2020). Another possible way of assimilation is through dietary exposure, as reported by Kakimoto et al. (2014), Kim et al. (2014), L'Homme et al. (2015) and Zacs et al (2021).

Some studies indeed reported the presence of DRCs in food for human consumption such as fish products in Japan (Kakimoto et al., 2012) and in some European states (Aznar-Alemany et al., 2017). Other food matrices were also reported to be contaminated in Korea (Kim et al., 2014), Lebanon (Abdel Malak et al., 2019), Belgium (L'Homme et al., 2015: Poma et al., 2018), Latvia (Zacs et al., 2021) and four African countries (Vaccher et al., 2020).

In foodstuffs from Belgium, DP was found in 5% of 1289 samples, and in particular in cheese, quail and pork eggs with average DP levels were 339, 637 and 331 pg/g ww, respectively) (Poma et al., 2018). In 175 samples of 35 different food products from the retail market in Korea, syn- and anti-DP were detected in 83.4 and 79.4% of the food product and the average DP value was between ND-170 pg/g ww (Kim et al., 2014), with a daily DP intake estimated in 11.2×103 pg/day, which was 3 orders of magnitude higher

than other declorans. In a study conducted in Lebanon of 58 food samples representing fatty food groups, the estimated daily dietary intake for the adult population had LB and UB limits of 1344 and 1718 pg/day for DP (Malak et al., 2019) .

Also in this case, the highest levels of DP are generally found in food products produced near the e-waste recycling site (Wang et al., 2013), in fact in a study comparing human exposure at a production facility and an e-waste facility in Southern China, high levels of DP were found in foodstuff produced in proximity to the e-waste recycling site and production facility (Wang et al., 2013). In another study from an e-waste recycling area in China, free-range chicken eggs harvested in 2010, 2013 and 2016 contained high levels of flame retardants, including DP (Huang et al., 2018).

1.3.4 Measurement methods for DRCs in food samples

Due to their distribution in environment and labware some precautions must be taken when processing samples. These precautions include for example the use of high purity solvents and furnace glassware (Sverko et al., 2011). It is also important to use procedural blanks to monitor the levels of contamination (which is not always adequately described and considered in some works) and adequate internal standards (not always commercialy easily available) in order to increase the robustness of the analytical data obtained. DRCs are lipophilic substances and therefore the extraction methods used are similar to those employed for non-dioxin-like polychlorinated biphenyls (NDL-PCBs). First it is necessary co-extract the DRCs and the fat matrix through the use of a specific solvent with a chemical and/or mechanical mechanism. Several methods are described in the literature, such as liquid-liquid extraction (LLE), solid-liquid extraction (SLE), solid phase extraction (SPE), Soxhlet extraction (SE) and pressurized liquid extraction (PLE), depending to the type of sample (Wang et al., 2016). SE and PLE are applied to solid sample (Wang et al., 2016), and generally the matrix is previously homogenized and freeze-dried. In particular, PLE is a type of automated, fast and economical extraction. The solid sample is placed in a stainless steel extraction cell and a solvent is then pumped into the cell at high pressure and high temperature, to speed up the extraction process. The liquid that passes through the cell and the sample is then transferred to a collection vial. Several factors can affect the effectiveness of PLE extraction:

- *Type of solvent*: It must be selective, capable of extracting the maximum amount of analyte and minimizing the extraction of other substances.

- *Temperature*: high temperatures decrease the viscosity of the solvent facilitating the penetration into the matrix and accelerating the extraction process.

- *High pressure*: high pressures allow the solvent to pass through the pores of the matrix, and this would not be possible at atmospheric pressure.

- *Extraction time*: adequate times are required for correct contact and penetration between solvent and matrix.

For the extraction, generally mixes of organic solvents are used which can be for example n-hexane/acetone (v/v: 1/1) (Hoh et al., 2006), or n-hexane/dichloromethane (v/v: 1/1). The extracts are generally purified before the instrumental analysis. To eliminate the interferents present in the matrix that could disturb the detection of the analytes (for example through the phenomenon of ion suppression) the sample must be purified before extraction. The most common methods of purification involve the use of silica gel column or multilayer column packed with acid and basic silica gel (Wang et al., 2016). Acid silica, activated with sulfuric acid, is used to eliminate and degrade lipids, while basic silica, activated by potassium hydroxide, eliminates acidic polar interferents. Finally, the addition of sodium sulfate in the silica columns is recommended to remove traces of water and ensure anhydrous conditions. For the elution of DRCs on silica gel column the most used solvent is n-hexane (possibly mixed with dichloromethane) (Wang et al., 2016). In addition, for biota samples such as fish, gel-permeation chromatography (GPC) it is commonly used to remove large molecules, protein and lipids from the extract (Kakimoto et al., 2012; Rjabova et al., 2016; Abdel Malak et al., 2018). GPC is a nondestructive methods, and is an excellent alternative to the concentrated sulfuric acid method, which can degrades some analytes of interest. For DRCs, the SX-3 Bio-Beads is generally used as the stationary phase, while the most widely used mobile phases are composed of ethylene/cyclohexane (v/v: 1/1) or hexane/dichloromethane (v/v: 1/1) mixtures. Separation and quantification of the DRCs is carried out in almost all the works by gas chromatography (GC) due to the apolarity of these compounds, coupled with mass spectrometry (MS). The most used MS approaches are electron capture negative ion (ECNI)-MS, negative chemical ionization

(NCI)-MS, NCI-MS/MS, and electron impact-high resolution mass spectrometry (EI-HRMS) (Hoh et al., 2006; Brasseur et al., 2016; Wang et al., 2016; Rjabova et al., 2018).

2. AIM

In the food industry, "contaminant" is defined as any substance added unintentionally to the food, but which is present in it as a result of the production process (including operations carried out in agriculture, livestock and veterinary medicine) and manufacturing processes, transformation, preparation, treatment, packaging, transport or storage of such food, or following environmental contamination. Environmental pollution is one of the main causes of food contamination, and the presence of non-biodegradable chemicals that are accumulated by humans through food chain is particularly concerned.

In recent years there has been a growing interest from the scientific community in a particular class of environmental pollutants, defined as "contaminants of emerging concern" (CECs) which includes a large group of chemicals that are released into the environment by human activities and causes known or suspected harmful effects on human health, but which are currently not monitored. Their presence in the environment varies from a few μg/L to a few ng/L and sometimes even lower, so until recently it was impossible to discover, detect and monitor these compounds at such low concentrations. Some of these contaminants are ubiquitous and persistent, and by polluting the air, water and soil they enter the food chain bioaccumulating and reaching humans. Although environmental and human toxicology has not yet been adequately studied for most of these compounds, it is believed that they may be potentially harmful to humans. Two important classes of emerging contaminants are PFAS and DRCs, which are also considered by the Stockholm Convention on Persistent Organic Pollution. Some of these substances are in fact listed in the annexes to the convention (or are under review by the POP Review Committee to become part of the substances listed in the convention) which establish measures to limit, reduce or eliminate the presence of these compounds in the environment, due to their harmful characteristics to humans and to the environment.

The aim of this thesis was to investigate these two categories of CECs, PFAS and DRC, in food of animal origin. The work was conduced in two different steps:

- The determination of PFAS was carried out at "Laboratorio di Chimica Analitica Bio-Agroalimentare (CABA-Lab) of the Biosciences and Biotechnology Service of the Department of Veterinary Medical Sciences (UNIBO) Ozzano dell'Emilia (BO), Italy. The purpose of this part of work was to investigate and compare PFAS

contamination in Italian eggs of commercial laying hens reared according to the systems provided by EU legislation (battery cage, barn, aviary system and organic) and in home produced eggs from backyard chickens.

Chicken eggs are an important part of the human diet and their consumption does not have any kind of limitations (ethical, religious, economic and environmental), but information on their contamination by PFAS is still very scarce, especially in Italy. Environmental contamination from PFAS can be widespread, due to the persistence characteristics of these substances or localized due to accidents or uncontrolled spills in highly industrialized areas. The issue has recently become famous in Italy, following high levels of contamination detected in a specific area of the Veneto Region, probably attributable to industrial spills.

In this study the mixture of the four PFASs (evaluated by the latest EFSA opinion) was considered: perfluoro-1-octanesulfonate (PFOS), perfluoro-n-octanoic-acid (PFOA), perfluoro-n-nonanoic acid (PFNA) and perfluoro-1-hexanesulfonate (PFHxS).

The analyses were performed by ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS).

The project is carried out in collaboration with the Department of Agro-Food Sciences and Technologies and developed in accordance with the Department's certified Quality Management System, PFASs-EGG 03-17/BSBT/CABA-Lab "Determination of PFAS in eggs ". All operations concerning the determination of PFAS in eggs have been conducted in accordance with the certified Quality Management System of the Department of Veterinary Medical Sciences.

- The determination of DRCs was carried out during the training period held abroad during the PhD, at and in collaboration with LABERCA **(LABoratoire d'Étude des Résidus et Contaminants dans les Aliments)** that is a Joint Research Unit of Nantes Atlantic College of Veterinary Medicine, Food Science and Engineering (Oniris) located in Nantes with INRAE (Institut national de recherche pour l'agriculture, l'alimentation et l'environnement) (France). Studies on DRCs contamination in food are still scarce. Most focus on food matrices such as fish, and often only for the purpose of environmental monitoring, and not to estimate human food intake. The foods of the other categories are poorly considered in the studies present in the literature and therefore the available data are few. For this reason, the

project involved the determination of DRCs on different categories of food. We decided to select only foods of animal origin, due to their high lipid content compared to vegetable foods, and therefore due to the lipophilicity of DRCs, they should have higher levels of contamination. The only vegetable foods sampled and analyzed were oils. The collaboration between the Italian and the French laboratory made it possible to compare the same food products from both countries. The DRCs researched in this study were six: syn-DP, anti-DP, Dec-601, Dec-602, Dec-603 and CP. Analyzes were performed by gas chromatography coupled to high resolution mass spectrometry using a Q Exactive GC Orbitrap. While the sample preparation method that will be described below had previously been used by LABERCA for research purpose, the instrumentation used was new and never tested for the analysis of DRCs on food. Therefore, the description of the analytical part of the work is still in a preliminary phase, and further tests will be necessary before optimizing all the instrumental parameters.

Part of the work described in this thesis has also been previously published in scientific journals (Gazzotti et al., 2021, Ghelli et al., 2019, Ghelli et al, 2021).

3. MATERIALS AND METHODS

3.1 PFASs IN ITALIAN COMMERCIAL AND HOME PRODUCED EGGS

3.1.1 Sample collection of commercial eggs

The egg samples were collected from Italian commercial laying hen farms in 2017. Sampling was based on rearing system variables of the eggs: organic, aviary system, battery cage and barn. A total of 132 eggs were collected, divided into 11 groups named in alphabetical order based on the variables listed in Table 3.1. After sampling, eggs were boiled and the yolks were separated from egg white. Therefore, for each group were created 4 pools each consisting of three homogenized yolks for a total of 44 samples analyzed. The pools were stored at -20°C until the analysis.

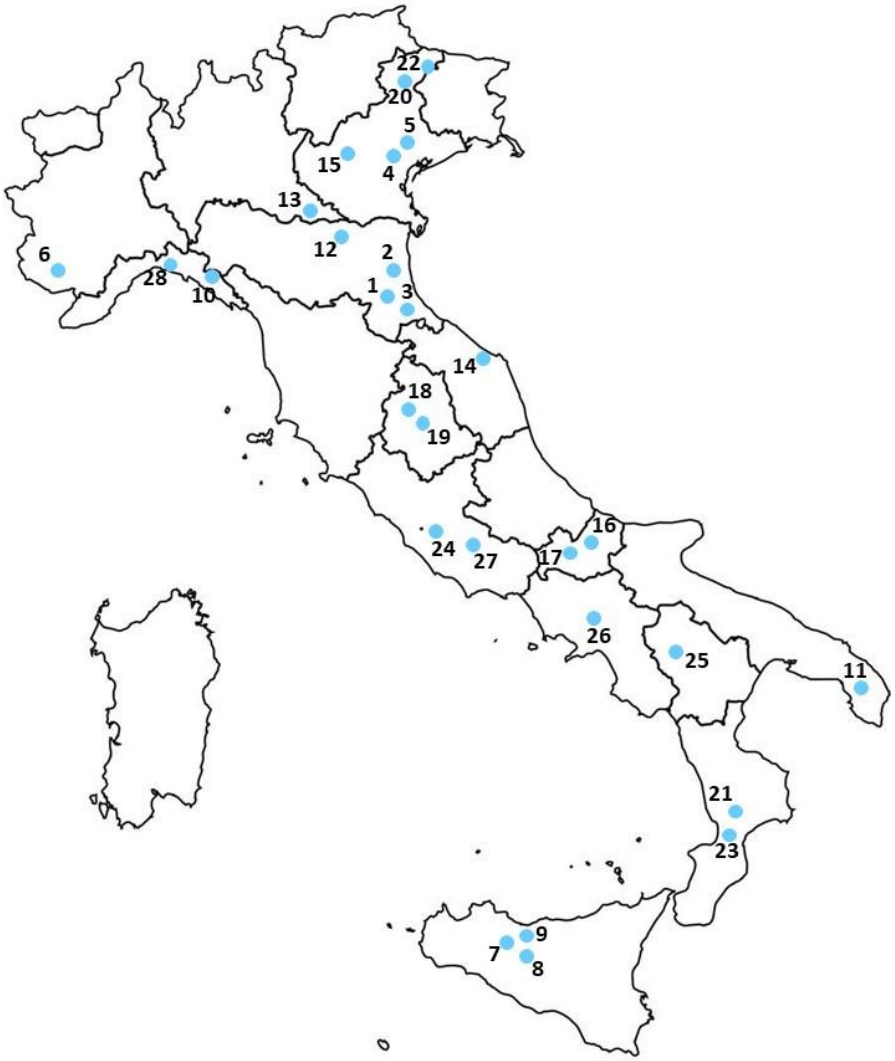
Table 3.1. Sampling of commercial eggs

Group	N° of pools formed	Rearing system
A	4	Barn
B	4	Organic
C	4	Battery cage
D	4	Barn
E	4	Battery cage
F	4	Aviary system
G	4	Aviary system
H	4	Organic
I	4	Battery cage
L	4	Organic
M	4	Barn

3.1.2 Sample collection of home produced eggs

The eggs were sampled in Italy during 2017 from domestic farms in the following regions: Piemonte, Lombardia, Liguria, Veneto, Trentino Alto Adige, Friuli Venezia Giulia, Emilia-Romagna, Marche, Umbria, Lazio, Molise, Campania, Basilicata, Puglia, Calabria and Sicilia. A total of 224 home produced egg samples were collected in 28 points distributed along the Italian peninsula, as shown in figure 3.1. For each sampling point, from 3 to 15 eggs were collected and divided to from 1-5 pools of two or three eggs for each group. The total number of pools formed is 86. After sampling, eggs were boiled and the yolks were separated from egg with and then the pools were stored at -20 °C until the analysis.

Figure 3.1. Geographical distribution of home produced eggs sampled



3.1.3 Reagents and Chemicals

To verify the method and quantify the samples, standards of PFOA, PFOS, PFNA and PFHxS at 50 mg/L (50 ppm) in methanol (purity> 98%) were used, purchased from Wellington (Guelph, Ontario, Canada). Also their respective labeled standards (IS), perfluoro–n– (1,2,3,4,5-$^{13}C_5$) nonanoic acid (M-PFNA), perfluoro–n– (1,2,3,4-$^{13}C_4$) octanoic acid (M-PFOA), perfluoro-1-hexane ($^{18}O_2$) sulfonate (M-PFHxS), sodium perfluoro-1-(1,2,3,4-$^{13}C_4$) octanesulfonate (M-PFOS) 50 mg/L (50 ppm) in methanol (purity> 98%) were purchased from Wellington (Guelph, Ontario, Canada).
The chemicals used for mass spectrometry analysis were LC-MS grade and included: Ultrapure water (18.2 MΩ/cm) obtained via a Human Power I lab water purification system (Human Corp., Seoul, South Korea), methanol, ammonium acetate, acetonitrile and formic acid all from Fluka (Honeywell).
The solvents used for the extraction of the samples were instead all of analytical grade and included distilled water, Acetonitrile from Merk (Darmstadt, Germany), Hydrochloric acid (37%) from Carlo Erba Reagents (Cornaredo, MI, Italy), Sodium hydroxide pellets (>98%) from Fluka (Honeywell) and Sodium acetate, Ammonium hydroxide (33%) and Methanol all from Sigma-Aldrich (St. Louis, MO, USA).
Oasis ™ WAX 3cc, 60 mg, 60 µm Solid Phase Extraction (SPE) cartridges (Waters Corporation, USA) were used to extract analytes from samples.
A stock solution at a concentration of 2 mg/L (2ppm) was prepared for each standard and its respective labeled by taking 1 ml of each standard and labeled standard at 50 ppm and diluting it with 25 ml methanol of LC-MS grade.
From these solutions, two working solutions were obtained in the following way

- *PFASs working solution* at a concentration of 50 ng/mL (50 ppb).
  25 µL of each standard at 2 ppm was taken (100 µL of total standard) and made up to volume with 900 µL of methanol LC-MS grade.

- *"IS working solution"* at a concentration of 50 ng/mL (50 ppb)
  25 µL of each labeled standard at 2 ng/µL was taken (100 µL of total labeled standard) and made up to volume with 900 µL of methanol LC-MS grade.

Each solution was prepared in polypropylene flasks and stored in a freezer at a temperature of -20 °C.

3.1.4 Sample Preparation

The samples were extracted referring to the Zafeiraki method (Zafeiraki et al., 2016), adapted according to our laboratory conditions and already used in our previously published works (Ghelli et al, 2019; Gazzotti et al., 2021). To avoid interference in the analysis of the samples, all glass laboratory materials were replaced with polypropylene tubes and vials, also avoiding the use of paper filters and Teflon ™ caps.

All the eggs were previously boiled for 10 minutes in distilled water, shelled and the egg whites were separated from the yolks. To form the pools, the yolks were homogenized with a mortar inside beaker and then transferred to a tube.

The PFAS were determined only in the yolk because earlier works in literature have shown how the PFAS tend to accumulate in the lipid part of the egg, and therefore to concentrate mainly in the yolk and to be present at negligible levels in the egg white (Zafeiraki et al., 2016).

For each sample 1 g of homogenized yolk was weighed, placed in a 50 mL polypropylene tube, to which 50 μL of "IS working solution" at 50 ng/mL were added for quantification. Subsequently, in each sample 2 mL of 200 mM sodium hydroxide were added for alkaline digestion and homogenized with Ultra-turrax for 1 minute. Then 10 mL of methanol were added for extraction and samples were agitated by magnetic stirrer for 30 minute at 250 rpm. To neutralize the solution, 150 μL of hydrochloric acid was added and the sample was centrifuged for 10 minutes at 9000 rpm. The supernatant was transferred to a new 50 mL tube, previously prepared with 25 mL of pure water. Each tube was vortexed for 1 minute. The extract was purified by SPE Oasis WAX (Weak Anionic eXchange) cartridges. Before loading the samples, the SPE Oasis ™ WAX 3cc, 60 mg cartridges were conditioned with 4 mL of methanol and 4 mL of pure water. The sample was then loaded into the cartridges and then washed with 4 mL of 25 mM sodium acetate solution at pH 4 with hydrochloric acid.

Before elution, vacuum was applied for 1 minute in order to completely eliminate the washing solutions. The eluate was obtained by pouring into the cartridges 2 ml of ammonium hydroxide in a 2% solution in acetonitrile: the sample collected in Eppendorf® was subsequently evaporated under nitrogen flow at a temperature of 45 °C.

After drying the samples completely, they were reconstituted with 300 μL of 20 mM ammonium acetate aqueous solution:methanol (90:10). Finally, the samples were vortexed

for 30 seconds and transferred to polypropylene vials for analysis with LC-MS/MS. The procedure has been schematized in the figure 3.2**.**

Figure 3.2. Diagram of the sample extraction process of PFAS in egg yolk.

3.1.5 Analytical conditions

The separation of the analytes was achieved using an Acquity ultra-performance liquid chromatographic system consisting of a binary pump, solvent degasser, autosampler and column heater fitted with a Waters BEH C18 column (1.7 μm, 2.1 x 50 mm) equipped with a guard column (Waters Corporation, Milford, MA, USA). To minimize the interferents of the matrix, an ACQUITY PFC Isolator Column 2.1 x 50mm (Waters Corporation, Milford, USA) was inserted into the UPLC pump, in order to block any traces of PFAS coming from the mobile phases used and thus avoid contamination and the consequent overestimates in quantification. The analysis was conducted under programmed conditions at a flow rate of 0.4 mL/min. The mobile phase consisted of two solutions A and B:

- **Phase A**: 20 mM ammonium acetate aqueous solution;
- **Phase B**: methanol.

The elution gradient started with 10% B for 1,50 min; followed by 1 min linear gradient to 80% B, then 1,50 min hold at 80% B, and return back to 10% B in 50 sec. The column was equilibrated during 1.10 prior to the next injection. The chromatographic separation gradient is shown in Table 3.3. The injection volume was set at 10 μL. The samples were kept at room temperature and the column was thermostated at a temperature of 40 °C.

Table 3.2. Analytical conditions of UPLC system

Mobile Phase A	20 mM ammonium acetate in water	
Mobile Phase B	Methanol.	
Gradient	**T 0$_{min}$**	90% A – 10% B
	T 1,5$_{min}$	90% A – 10% B
	T 2,5$_{min}$	20% A – 80% B
	T 4$_{min}$	20% A – 80% B
	T 4,5$_{min}$	90% A – 10% B
	T 6$_{min}$	90% A – 10% B
Flow rate	0,4 mL/min	
Volume of injection	10 µL	
Column temperature	40 °C	

The autosampler needle was washed prior to each single injection with the following solutions, solutions (LC-MS grade):

- **Weak solution**: Methanol: Acetonitrile: ultrapure water (40:30:30 v/v);
- **Strong solution**: 20 mM ammonium acetate in ultrapure water: Methanol (80:20 v/v).

The detector used was the Quattro Premiere XE mass spectrometer, equipped with a triple quadrupole instrument with an ESCI™ Multi-Mode Ionization Source (Waters Corporation) and operating in negative electrospray ionization (ESI−) mode.
The instrument worked in multiple reaction monitoring mode (MRM) and the two transitions for each analyte and their respective IS were monitored, as shown in Table 3.4. The most intense transition between the two monitored was used for the quantification, with the exception of the one identified for PFOS.

Table 3.3. Scheme of monitored transitions for PFAS and labeled PFAS, cone voltages and collision energies. The quantified ions are bold in the central column

Compound	Precursor Ion (m/z)	Product Ions (m/z)	Cone Voltage (kV)	Collision Energy (eV)
PFOS	498.50	**99.10**	50	35
		80.20	50	40
PFHxS	398.60	**99.10**	52	35
		80.20	52	38
PFNA	462.50	**419.00**	12	10
		219.10	12	16
PFOA	412.60	**368.90**	12	9
		169.00	12	17
M-PFOS	502.50	**99.10**	50	35
		80.20	50	40
M-PFHxS	402.60	**103.10**	55	32
		84.20	55	35
M-PFNA	467.50	**423.00**	12	10
		219.10	12	16
M-PFOA	416.50	**372.00**	12	10
		169.00	12	17

Nitrogen was used as desolvation gas and Argon was employed as collision gas. The parameters set for the mass spectrometer were:

- Desolvation gas flow 700 L/hr
- Collision gas flow 0.35 mL/min

- Cone gas 100 flow L/hr
- Capillary voltage was 2.0 kV
- Extractor voltage 2.00 V
- Desolvation temperature 450°C
- Source temperature 150°C

Data acquisition and processing was performed using Mass Lynx 4.1 software (Waters Corp.).

3.1.6 Calibration and validation

The isotope dilution technique, using labeled compounds (M-PFOA, M-PFHxS, M-PFOS and M-PFNA), ensured a higher level of accuracy of the data, as these compounds have a chemical structure identical to that of the target molecules, and therefore also the same behavior both during the preparation of the sample and during the analyzes. Their use therefore allowed to compensate for any phenomena of ion suppression and variability in the recovery of the analytes providing a sufficient correction of the response for a reliable quantification.

A matrix-matched calibration curves were prepared each day of analysis. 1 g of blank yolk was spiked with appropriate amounts of the "PFASs working solution" to obtain 5 levels of concentration (in the range between 0 and 10 µg/kg) and 50 µL of the "IS working solution" (50 ng/mL) according to the scheme shown in the Table 3.5. Quality control (QC) samples were prepared at three different levels of concentration (0.5, 2, and 5 µg/kg) in four replicates proving method's efficiency in terms of accuracy.

Table 3.4. Scheme of the fortifications used for the calibration curve of PFAS

Concentration level (ng/mL)	PFAS Working solution (50 ng/mL)	IS Working solution (50 ng/mL)
0	0 µL	50 µL
0,5	10 µL	50 µL
1	20 µL	50 µL
5	100 µL	50 µL
10	200 µL	50 µL

3.2 DRCs IN FOODS OF ANIMAL ORIGINS PURCHASED FROM ITALIAN AND FRENCH LARGE-SCALE RETAILERS.

3.2.1 Sample collection

The samples of the present study were purchased during October and November 2019 in French and Italian supermarkets. A total of 30 food samples (almost all of animal origin) were collected for each country, divided in the following groups: meat and meat products (n=9), eggs and egg products (n=3), fish and other seafood (n=7), milk and dairy products (n=5), animal and vegetable fats and oil (n=6). We tried to collect the same types of products, where possible, considering any differences in eating habits that exist between the two nations. Detailed information of the Italian and French sampled products is shown respectively in the table 3.5 and 3.6. Differences consist in one type of fish (samples number 14), one of cheese (samples number 24) and one of oil (samples number 28), which are different between Italy and France. The remaining samples are the same for both, in order to facilitate the comparison of the results. Excepted for the vegetable fats and oil (that were purified directly) the samples were weighted, freeze-dried (for better sample storage and extraction efficiency), homogenized with stainless steel blender, weighed again and then stored at -20 °C until chemical analysis.

Table 3.5. Details of Italian food samples

Sample name		Food category	Food details
		Meat and meat products	
IT_1	*Beef*	Livestock meat	Beef fillet
IT_2		Livestock meat	Beef steak
IT_3		Livestock meat	Beef hamburger
IT_4	*Pork*	Livestock meat	Pork loin
IT_5		Preserved meat	Baked ham
IT_6		Sausages	Pork sausage
IT_7	*Poultry*	Poultry	Chicken thigh
IT_8		Poultry	Chicken breast
IT_9		Poultry	Chicken leg
		Eggs and egg products	
IT_10	*Eggs*	Eggs, fresh	Organic eggs
IT_11		Eggs, fresh	Free range eggs
IT_12		Eggs, fresh	Barn eggs
		Fish and other seafood	
IT_13	*Fish*	Fish meat	Salmon (*Salmo salar*)

IT_14		Fish meat	Sea bass (*Dicentrarchus labrax*)
IT_15		Fish meat	Sea bream (*Sparus aurata*)
IT_16		Fish meat	Cod (*Gadus morhua*)
IT_17		Fish meat	Canned tuna
IT_18	*Seafood*	Crustaceans	Prawn (*Penaeus vannamei*)
IT_19		Water molluscs	Mussels (*Mytilus galloprovincialis*)
		Milk and dairy products	
IT_20	*Milk*	Liquid milk	Whole UHT milk
IT_21		Liquid milk	Low-fat UHT milk
IT_22	*Cheese*	Cheese	Emmental cheese
IT_23		Cheese	Mozzarella cheese
IT_24		Cheese	Grana Padano cheese
		Animal and vegetable fats and oil	
IT_25	*Oil*	Vegetable oil	Extravirgin olive oil
IT_26		Vegetable oil	Olive oil
IT_27		Vegetable oil	Sunflower oil
IT_28		Vegetable oil	Corn oil
IT_29	*Animal fat*	Animal fat	Butter
IT_30	*Vegetable fat*	Margarine and similar products	Margarine

Table 3.6. Details of French food samples

Sample name		Food category	Food details
		Meat and meat products	
FR_1	*Beef*	Livestock meat	Beef fillet
FR_2		Livestock meat	Beef steak
FR_3		Livestock meat	Beef hamburger
FR_4	*Pork*	Livestock meat	Pork loin
FR_5		Preserved meat	Baked ham
FR_6		Sausages	Pork sausage
FR_7	*Poultry*	Poultry	Chicken thigh
FR_8		Poultry	Chicken breast
FR_9		Poultry	Chicken leg
		Eggs and egg products	
FR_10	*Eggs*	Eggs, fresh	Organic eggs
FR_11		Eggs, fresh	Free range eggs
FR_12		Eggs, fresh	Barn eggs
		Fish and other seafood	
FR_13	*Fish*	Fish meat	Salmon
FR_14		Fish meat	Atlantic wolffish (*Anarhichas lupus*)

FR_15		Fish meat	Sea bream (*Sparus aurata*)
FR_16		Fish meat	Salted cod
FR_17		Fish meat	Canned tuna
FR_18	*Seafood*	Crustaceans	Prawn (*Penaeus vannamei*)
FR_19		Water molluscs	Mussels (*Mytilus galloprovincialis*)
		Milk and dairy products	
FR_20	*Milk*	Liquid milk	Whole UHT milk
FR_21		Liquid milk	Low-fat UHT milk
FR_22	*Cheese*	Cheese	Emmental cheese
FR_23		Cheese	Mozzarella cheese
FR_24		Cheese	Camembert cheese
		Animal and vegetable fats and oil	
FR_25	*Oil*	Vegetable oil	Extravirgin olive oil
FR_26		Vegetable oil	Olive oil
FR_27		Vegetable oil	Sunflower oil
FR_28		Vegetable oil	Rapeseed oil
FR_29	*Animal fat*	Animal fat	Butter
FR_30	*Vegetable fat*	Margarine and similar products	Margarine

3.2.2 Reagents and Chemicals

For the calibration curve were used standard solutions of native syn-Dechlorane Plus (DP), anti-DP, Dec-601, -602, -603 and Chlordene Plus (CP), provided by Wellington Laboratories (Guelph, Ontario, Canada). As internal standards (IS) were used reference solutions of labelled $^{13}C_{10}$-Dec-602, $^{13}C_{10}$-syn-DP and $^{13}C_{10}$-anti-DP provided by Cambridge Isotope Laboratories (Andover, MA, USA) and used according to the isotopic dilution method. The reference solution of $^{13}C_{12}$-PCB-194, also supplied by the Cambridge Isotope Laboratories was used as a recovery standard (RS). Silica gel (70/230 mesh) was obtained from Fluka (Buchs, Switzerland), sodium sulphate from Merck (Darmstadt, Germany). Sodium hydroxide 1 N was purchased from Reagecon (Clare, Ireland) and sulphuric acid (98%) from Panreac (Barcelona, Spain). Toluene, acetone, ethyl acetate, cyclohexane and *n*-hexane were provided by LGC Promochem (Picograde®, Wesel, Germany) and dichloromethane by Biosolve (Valkenswaard, The Netherlands).

The neutral silica was prepared by purifying 1 kg of silica with 1 L of methanol, 1 L of dichloromethane and 1 L of hexane in succession. The obtained silica was then completely dried in an oven at 130 °C for 48 hours. From this silica the basic one at 33% was then

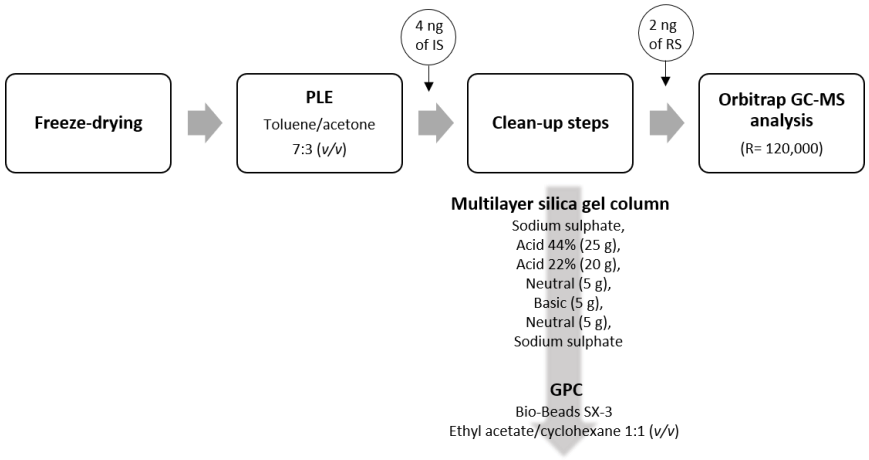
prepared using sodium hydroxide and the acidic ones at 22% and 44% using sulphuric acid. The standard solutions were prepared by taking an adequate amount of $^{13}C_{10}$-Dec-602, $^{13}C_{10}$-syn-DP and $^{13}C_{10}$-anti-DP solutions and diluted with toluene to create a 100 ng/µL mix IS solution. The same operation was done with the $^{13}C_{12}$-PCB-194 solution and with the native standard solutions in order to obtain a 100 ng/µL RS solution and a 100 ng/µL mix native standard solution in toluene.

3.2.3 Sample Preparation

The analysis was conducted according to the previously developed method, which is described in the works of Abdel Malak et al. (2018, 2019), on a range of food products of animal origin. This method comes from an adaptation of what is present in the literature (Wang et al., 2016), with some slight modifications, and is commonly used in research unit for analysis of contaminants of this type. Four grams of previously lyophilized sample were extracted by pressurized liquid extraction (PLE, Büchi, SpeedExtractor, E_914) with diatomaceous earth using 120 mL of a solvent mixture composed of toluene/acetone 7:3 (v/v). A total of 3 consecutive extraction cycles at 100 bar and 120 ° C were carried out (for a total of 50 minutes). For the animal and vegetable fats and oil samples instead, as previously mentioned, 1 g were directly weighed. The samples were collected in round-bottomed glass flasks whose weight was previously noted and then fortified with 40 µL of IS solution at 100 ng/µL (4 ng), composed with a $^{13}C_{10}$-labelled syn-DP, anti-DP and Dec-602 mixture. The samples were concentrated using rotary evaporator and then completely dried under a gentle stream of nitrogen. Finally, the lipid content was gravimetrically determined. The dry residue was then reconstituted with 15 mL of *n*-hexane and two steps of purification were applied to the sample. The first consisted of a clean-up using a column made up of several layers of silica. The column was composed of an upper layer of dry Na_2SO_4, below there were 25 g of acidic silica gel 44% (with sulphuric acid), 20 g of acidic silica gel 22% (with sulphuric acid), 5 g of neutral silica gel, 5 g of basic silica gel (NaOH 1 N), 5 g of neutral silica gel and on the bottom dry Na_2SO_4 again. Before loading the sample, theccolumn was conditioned with 150 mL of *n*-hexane. The sample extract was then loaded and DCRs were eluted with 100 mL of *n*-hexane, concentrated to dryness and reconstituted in 500 µL of a mixture of ethyl acetate/cyclohexane 1:1 (v/v), in order to be injected in the Gel Permeation Chromatography (GPC) system for the second step of purification. The column used (58 cm - 24.4 mm) was packed with Bio-Beads SX-3 (Bio-

Rad, Philadelphia, PA) and worked on a flow rate of 5 mL/min. The collected fractions equal to 50 mL (20.6- 30.6 min) were concentrated using a rotary evaporator and the residual solvent was removed under a gentle stream of nitrogen. The residue was reconstituted in 20 µL of RS solution at 100 ng/µL (2 ng) before the instrumental analysis. The sample preparation procedure is schematized in figure 3.3.

Figure 3.3. Diagram of the sample extraction process of DRCs in food.



3.2.4 Analytical conditions

Separation and detection of analytes in the purified extracts was performed with a gas chromatography (GC) coupled to high resolution mass spectrometry (MS) (Q Exactive GC Orbitrap, Thermo Fischer Scientific, San José, CA, USA), fitted with an electron impact ionisation source. Two microliters (toluene) were injected in the splitless mode at 300 °C. Helium was used as carrier gas flowing at 1 mL/min in a HT8-PCB column (30 m × 0.25 mm, SGE Analytical Science, Ringwood, Australia), which allows an optimized separation of DRCs (Abdel Malak et al., 2018). The oven temperature program started at 100 °C (2 min), rose to 280 °C at 30 °C/min, then ramped to 325 °C at 5 °C/min (6 min).

Table 3.7. Analytical conditions of GC system

Carrier gas	Helium
Flow rate	1 mL/min
Mode of injection	splitless
Volume of injection	2 µL

Temperature of injection	300 °C

Table 3.8. Oven temperature program of GC

Oven temperature program	**T 0$_{min}$**	100 °C
	T 2$_{min}$	100 °C
	T 8$_{min}$	280 °C
	T 16$_{min}$	325 °C
	T 22$_{min}$	325 °C

Auxiliary temperature was set at 325 °C and electron energy at 70 eV. Data were acquired in the positive mode over the *m/z* range [200–700] at a nominal resolving power of 120,000 full width at half-maximum at *m/z* 200. Trace analysis of targeted DRCs (*anti*-DP, *syn*-DP, Dec-601, Dec-602, Dec-604 and CP) was performed through isotopic dilution using $^{13}C_{10}$-*syn*-DP, $^{13}C_{10}$-*anti*-DP and $^{13}C_{10}$-Dec-602 as internal standards (4 ng, each), and $^{13}C_{12}$-PCB-194 as recovery standard (2 ng). Diagnostic extracted ion chromatograms are described Table 3.4. Identification was performed according criteria laid down in Commission Decision 2002/657/EC, more precisely pending at least two signals complying ion ratio tolerances. Data acquisition and processing was performed using Xcalibur software (Thermo Fischer Scientific, San Jose, CA, USA.)

Table 3.9. Diagnostic signal parameters for the analysis of targeted DRCs by Q Exactive GC Orbitrap. Theoretical ion ratios were determined according to enviPat Web 2.4 (https://www.envipat.eawag.ch/index.php, Loos et al., 2015) for isotopologues.

Target analyte	Ion	Quantifier Ion (m/z)	Qualifier Ion (m/z)	Internal standard
***Syn*-DP**	$[C_5Cl_6]^+$	271.80962	273.80667	$^{13}C_{10}$-*syn*-DP
***Anti*-DP**	$[C_5Cl_6]^+$	271.80962	273.80667	$^{13}C_{10}$-*anti*-DP
Dec-601	$[C_5Cl_6]^+$	271.80962	273.80667	$^{13}C_{10}$-*syn*-DP

Dec-602	$[C_5Cl_6]^+$	271.80962	273.80667	$^{13}C_{10}$-Dec-602
Dec-603	$[C_7H_2Cl_5]^+$	262.85641	264.85346	$^{13}C_{10}$-Dec-602
CP	$[C_5Cl_6]^+$	271.80962	273.80667	$^{13}C_{10}$-Dec-602
$^{13}C_{10}$-DP, $^{13}C_{10}$-Dec-602	$[^{13}C_5Cl_6]^+$	276.82639	278.82344	-
$^{13}C_{12}$-PCB-194	$[^{13}C_{12}H_2Cl_8]^+$	441.80028	439.80323	-

3.2.5 Quality assurance and quality control (QA/QC)

Maximum attention has been paid to procedural contamination. Glassware was heated in a muffle oven at 400 °C for at least 4 h, or, when it was not possible, rinsed with dichloromethane. A total of 9 procedural blanks were processed starting from the extraction step, and treated in the same manner as food samples. If procedural contamination was observed, a limit of quantification (LOQ) was set at mean plus 3 times the standard deviation of the procedural blank. When no procedural contamination was observed, a limit detection (LOD) was defined as 3 times to noise background. A single sequence including all samples and a standards calibration curve was operated. The calibration curve was obtained by adding an adequate amount of DRC native standard solution mix (from the starting solution at 100 ng/µL, three successive dilution intermediate solutions were prepared, at concentrations of 10 ng/µL, 1 ng/µL and 0.1 ng/µL, used for the different points of the calibration curve) to obtain 8 concentrations, as prepared, and 30 µL of this was added to each point of the curve (300 pg). Finally, the points of the curve were reconstituted in 15 µL of RS solution at 20 ng/µL (300 pg), obtained by diluting the RS solution 100 ng/µL in toluene.

Table 3.10. Scheme of the fortifications used for the calibration curve of DRCs.

Concentration level (ng/µL)	IS solution (10 ng/µL)	Native mix standard solution	RS solution (20 ng/µL)
0	30 µL	-	15 µL
1	30 µL	10 µL (0,1 ng/µL)	15 µL
3	30 µL	30 µL (0,1 ng/µL)	15 µL
10	30 µL	10 µL (1 ng/µL)	15 µL

30	30 µL	30 µL (1 ng/µL)	15 µL
100	30 µL	10 µL (10 ng/µL)	15 µL
300	30 µL	30 µL (10 ng/µL)	15 µL
1000	30 µL	10 µL (100 ng/µL)	15 µL

4. RESULTS AND DISCUSSIONS

4.1 PFASs IN ITALIAN COMMERCIAL AND HOME PRODUCED EGGS

Chicken eggs are a non-negligible part of the human diet and are a possible source of PFAS and POPs in general, and the level of contamination may depend on the chickens breeding system (Pajurek et al., 2019). The Italian consumption of eggs is equal to 13.4 kg per capita, slightly lower than the European average. In practice, every Italian eats a total of about 214 eggs per year, between direct and indirect consumption. About 40% of the product is used in the food industry in the form of egg products while the remaining 60% is consumed as whole eggs (ISMEA, 2019). Following the sensitivity of many customers, the large-scale retail trade has decided to stop or drastically decrease the sale of eggs from cage farming, increasing the assortment for those produced by free-range hens. Currently in Italy, free-range eggs hold the main share (45%) and between 2017 and 2018 they increased volumes by 28% (ISMEA, 2019). In addition to commercial laying hen eggs, many people consume barnyard eggs; these chickens are often free to stay outdoors and collect small insects from the ground. These habits may represent a route of exposure to environmental pollutants, such as PFAS, for chickens and their products (Pajurek et al., 2019; Zafeiraki et al., 2016).

4.1.1 Validation of the analytical method

The analysis of the injected matrix-matched calibration curves shows the good linearity of the method, with linear regression coefficient values (R^2) values always greater than 0.99. The protocol was therefore validated in accordance with current European guidelines set by Decision 2002/657/EC (European Commission, 2002). The specificity of the method was showed by the absence of potential interferences around the retention times of the analytes in the chromatograms of the non-contaminated yolk samples. Maximum relative standard deviation to the mean (CV%) of Quality control (QC) samples ranged from 9% to 19% and trueness (relative difference between the measured mean value and the spiked concentration) was always lower than 10%. Limits of quantification (LOQs) and limits of detection (LODs) of the method, defined as the concentrations providing a chromatographic signal with a signal-to-noise (S/N) ratio equal to 10 and 3 respectively, were set to 0.25 µg/kg and 0.1 µg/kg for all analytes.

4.1.2 Results obtained in commercial eggs

From the data obtained on commercial egg samples analyzed, shown in Table 4.1, it is immediately evident that in all the samples the level of each analyte was lower than the LOQ of 0.25 ng/mL, with the exception of two samples belonging to group A, obtained from barn, which showed contamination of PFOS in one sample and PFHxS in the other, both at the level of 0.4 µg/kg. Levels of PFOA, PFNA and PFHxS, between the LOD (0.1 ng/g) and LOQ, were found in the 15.9% of sample. PFOS, even if it was detected at a quantifiable level, was present in only 1 sample out of 48, while many studies report that PFOS and PFAS are the most frequent and abundant (Zafeiraki et al., 2016). In the samples analyzed, however, the most frequently analytes found were PFOS and PFHxS (as shown in Table 4.2), but with a detection frequency of 1% in both cases and therefore not significantly.

Table 4.1. Results of PFASs contamination level in analyzed commercial eggs. Traces: value between limit of detection (LOD) and limit of quantification (LOQ).

Group	Pool n°	n° of yolks	PFOA (µg/kg)	PFHxS (µg/kg)	PFOS (µg/kg)	PFNA (µg/kg)
A	1	3	traces	-	0.40	-
	2	3	traces	traces	-	-
	3	3	-	0.40	-	-
	4	3	-	-	-	-
B	5	3	-	-	-	-
	6	3	traces	-	-	-
	7	3	-	-	-	-
	8	3	-	traces	-	-
C	9	3	-	-	-	-
	10	3	-	-	-	traces
	11	3	-	-	-	-
	12	3	-	-	-	-
D	12	3	-	-	-	-
	14	3	-	-	-	-
	15	3	-	-	-	-
	16	3	-	-	-	traces
E	17	3	-	-	-	-
	18	3	-	-	-	-
	19	3	-	-	-	-
	20	3	-	-	-	-
F	21	3	-	-	-	-
	22	3	-	-	-	-
	23	3	-	-	-	-

	24	3	-	-	-	-
G	25	3	-	-	-	-
	26	3	-	-	-	-
	27	3	-	-	-	-
	28	3	-	-	-	-
H	29	3	-	-	-	-
	30	3	-	-	-	-
	31	3	-	-	-	-
	32	3	-	-	-	-
I	33	3	-	-	-	-
	34	3	traces	traces	-	-
	35	3	-	-	-	-
	36	3	-	-	-	-
L	37	3	-	-	-	-
	38	3	-	-	-	-
	39	3	-	-	-	-
	40	3	-	-	-	-
M	41	3	-	-	-	-
	42	3	-	-	-	-
	43	3	-	-	-	-
	44	3	-	-	-	-

Table 4.2. Range of detection, mean value measured above the LOQ and frequency of detection of PFAS analyzed in commercial eggs.

PFAS	Range commercial eggs (µg/kg)	Mean value measured above the LOQ (µg/kg)	Detection frequency
PFNA	<0.10-<0,25	<0.10	0
PFOS	<0.10-0.40	0.40	1%
PFHxS	<0.10-0.40	0.40	1%
PFOA	<0.10-<0.25	<0.10	0

The origin of the sampled eggs according to the rearing system was as follows: 27% organic, 27% cage battery, 27% barn and 19% aviary system. We would have expected slightly higher levels of contamination in barn and organic systems, due to the contact with the soil that the hens have, and therefore a greater exposure to PFAS present in the environment; but the levels of contamination were so limited that no significant differences emerged. This very low uniform distribution of PFAS in commercial eggs is in agreement

with the few data reported in the literature by Zafeiraki's study on Greek and Dutch commercial eggs (Zafeiraki et al., 2016).
Furthermore, the low contamination of commercial eggs contrasts the higher levels found in home produced eggs as reported in the literature (D'Hollander et al., 2011; Zafeiraki et al., 2016) and as also emerged from the domestic egg samples analyzed in this thesis.

4.1.3 Results obtained in home produced eggs from home produced eggs

The results obtained to home produced eggs from backyard chickens in Italy, reported in table 4.3, show general levels of PFAS contamination much more widespread and at much higher concentrations than commercial eggs.

Table 4.3. Results of PFASs contamination level in analyzed home produced eggs. Traces: value between limit of detection (LOD) and limit of quantification (LOQ).

Sample point n°	Pool n°	n° of yolks	PFOA (µg/kg)	PFHxS (µg/kg)	PFOS (µg/kg)	PFNA (µg/kg)
1	1	2	traces	-	0.90	-
	2	2	traces	traces	1.07	traces
	3	2	-	-	0.64	traces
	4	2	traces	-	1.32	-
	5	2	traces	traces	traces	traces
2	6	2	-	-	traces	-
	7	2	-	traces	traces	-
	8	2	traces	-	-	-
	9	2	-	-	traces	-
	10	2	-	0.48	-	-
3	11	2	-	0.50	1.76	-
	12	2	traces	-	2.30	traces
	13	2	traces	-	1.84	-
	14	2	-	traces	1.28	traces
	15	2	-	traces	2.23	-
4	16	3	-	-	1.76	traces
	17	3	-	-	1.05	traces
	18	3	-	-	0.92	traces
	19	2	-	-	-	traces
	20	2	traces	-	1.51	traces
5	21	3	-	0.5	-	traces

	22	3	traces	-	-	traces
	23	3	traces	-	traces	traces
	24	3	-	traces	-	-
	25	3	-	-	0.7	traces
6	26	3	-	-	-	-
	27	3	-	-	-	-
7	28	3	-	-	-	-
	29	3	-	-	-	-
8	30	3	-	-	-	-
	31	3	-	traces	-	-
9	32	3	-	-	1.12	traces
	33	3	-	traces	1.60	traces
10	34	3	-	traces	traces	-
	35	3	-	-	1.36	-
11	36	2	-	traces	traces	-
	37	3	-	traces	traces	-
12	38	3	-	traces	0.95	-
	39	3	traces	0.46	3.47	-
13	40	2	-	-	0.64	-
	41	2	-	-	1.52	traces
14	42	3	traces	-	2.64	0.47
	43	3	traces	-	2.23	0.45
	44	3	-	-	2.01	traces
	45	3	-	-	1.29	-
15	46	3	-	-	1.09	-
	47	3	traces	-	1.45	traces
	48	3	0.62	-	2.82	1.20
	49	2	-	-	1.29	-
16	50	3	-	-	-	-
	51	3	-	-	-	-
	52	3	-	-	traces	-
	53	3	-	-	-	-
17	54	3	-	-	-	-
	55	3	-	-	-	-
	56	3	-	-	-	-
	57	3	-	-	-	-
18	58	3	-	-	0.60	traces
	59	3	-	-	0.37	traces
	60	3	-	-	traces	-
	61	3	-	-	-	-
19	62	3	-	-	-	-
	63	3	-	-	-	-

	64	3	-	-	-	-
	65	3	traces	traces	0.25	-
20	66	2	-	-	-	-
21	67	2	-	-	traces	traces
	68	2	-	traces	0.40	traces
22	69	3	-	-	-	-
	70	3	-	-	-	-
23	71	3	-	-	1.30	0.32
	72	3	-	-	0.82	traces
24	73	3	-	-	0.27	-
	74	3	-	-	traces	-
25	75	3	-	-	0.39	-
	76	3	-	-	-	-
26	77	2	-	-	1.38	traces
	78	2	-	traces	0.92	-
27	79	2	-	-	-	-
	80	2	-	-	-	-
	81	2	-	-	-	-
	82	2	-	-	-	-
28	83	2	traces	-	7,70	traces
	84	2	-	-	25,90	0,50
	85	2	-	-	16,30	traces
	86	2	-	0,90	6,50	-

As shown in table 4.4, in the analyzed samples the most abundant and widespread analyte was PFOS that was detected in 64% of pools and quantified in 51 % of them. PFOS contamination was present on 21 sampling sites out of 28 with average value of 2,45 µg/kg. Both PFNA and PFHxS were present in 13 different sampling sites along the peninsula and quantified in 6% of pools with a medium value of 0,6 µg/kg, but PFNA was detected in traces in 37% of the pools and PFHxS only in 23%; this shows a more sporadic and less homogeneous distribution of PFHxS. The least widespread and abundant analyte was PFOA, it was detected in only 20% of pools and quantified only in a pool and therefore in a single site.

Table 4.4. Range of detection, mean value measured above the LoQ and frequency of detection for PFAS analyzed in 86 pools from 28 sampling site.

PFAS	Range domestic eggs(µg/kg)	Mean value measured above the LOQ (µg/kg)	Detection frequency
PFNA	<0.10-1.20	0.60	37%
PFOS	<0.10-25.90	2.45	64%
PFHxS	<0.10-0.90	0.60	23%
PFOA	<0.10-0.60	0.60	20%

It must be considered that the eggs from the sampling sites 27 and 28 come from chickens reared for ornamental purposes, and therefore they eggs are not intended for human consumption. Because the values of the sampling site 28, in particular for the PFOS, are much higher than the other samples, it was decided to non included in the statistical analyzes of the following tables and figures present in this thesis. Although sampling site 27 did not shown detectable levels of any PFAS, it was equally excluded from the statistical analysis for the same reason. Therefore, the total samples evaluated for comparison of results refer to 78 pools and 26 sampling sites. Considering only these samples, the statistics differ slightly, as shown in table 4.5.

Table 4.5. Percentage of positive samples, non-quantifiable samples and quantifiable samples for PFAS analyzed in 76 pools from the 26 sampling sites considered.

	Positive samples (>LOD)		Non-quantifiable samples (LOD>X>LOQ)	Quantifiable samples (X>LOQ)				
	N°	%	N°	N°	%	Range µg/kg	Mean value	Standard deviation
PFOS	51	65%	12	39	50%	0.25-3.50	1.32	0.73
PFOA	16	21%	15	1	1%	0.25-0.60	0.60	-
PFNA	29	37%	25	4	5%	0.25-1.20	0.63	0.39
PFHxS	19	24%	15	4	5%	0.25-0.50	0.50	0.00

The most modified values with only 26 sampling points considered are average value of PFOS, that now is 1.32 µg/kg and average value of PFNA, that now is equal to 0,50 µg/kg. This results are in according to other study conducted in home produced eggs in Greece (Zafeiraki et al., 2016) and Belgium (D'Hollander et al., 2011), that reporting similar levels

of these class of contaminants. In the Zafeiraki work (2016) the mean values for PFOS range from 1.1-3.5 µg/kg, for PFOA 0.5-1.1 µg/kg, for PFNA 0.9-0.8 µg/kg and for PFHxS <0.5-1.1 µg/kg.

Regarding the distribution of contamination on the Italian territory, table 4.6 compares the levels of contamination measured in the samples collected in northern and southern regions of Italy. The LB was obtained by assigning a value of zero to all samples reported as lower than the LOD (<LOD) or LOQ (<LOQ). The UB was obtained by assigning the numerical value of LOD (0.10 µg/kg) to values reported as <LOD and LOQ (0.25 µg/kg) to values reported as <LOQ.

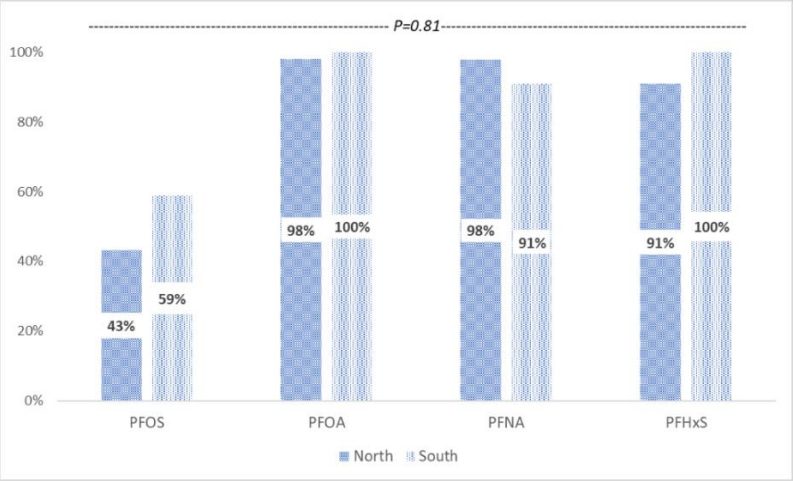
Table 4.6. Concentrations in ppb (µg/kg) of different PFAS in home produced eggs from backyard chickens collected in either northern or southern regions of Italy.

	Lower Bound (LB)		**Upper Bound (UB)**		***P*-value LB**	***P*-value UB**
	North	South	North	South		
PFOS	0.793 ± 0.13	0.486 ± 0.13	0.860 ± 0.12	0.567 ± 0.12	0.11	0.07
PFOA	0.014 ± 0.01	0.000 ± 0.00	0.152 ± 0.01	0.113 ± 0.01	0.38	0.02
PFNA	0.027 ± 0.03	0.038 ± 0.02	0.186 ± 0.03	0.160 ± 0.02	0.21	0.34
PFHxS	0.045 ± 0.02	0.000 ± 0.00	0.164 ± 0.02	0.131 ± 0.01	0.07	0.39
Σ PFAS	0.880 ± 0.16	0.524 ± 0.14	1.363 ± 0.15	0.971 ± 0.14	0.07	0.01

From this comparison, the most significant difference is represented by the total concentration of the mixture of the 4 PFAS, which is higher in the regions of northern Italy, both with the UB and LB approaches. A possible explanation is the greater concentration of industrial settlements in the regions of northern Italy.

Figure 4.1 compares the percentage of data considered Left Censored (below the limit of detection) for each PFAS, between the regions of northern and southern Italy.

Figure 4.1. Frequencies distribution of left censored (LC) data regarding the presence of different PFAS in home produced eggs from backyard chickens collected in either northern or southern regions of Italy.



The figure evidence how PFOS, PFOA and PSHxS are more frequently detected in the northern regions. PFNA instead, was most often found in samples from southern Italy.

4.1.4 Comparison between commercial eggs and eggs from backyard chickens

The direct comparison between data refer to eggs from commercial laying hens and backyard chickens in Italy showed in table 4.7 allowed to highlight statistically significant differences (P-value <0.01) for the total concentration of the mixture of the 4 PFAS and for PFOS for both LB and UB values and for PFNA for UB values.

Table 4.7. Comparison of the mean levels in ppb (µg/kg) of PFOS, PFOA, PFNA and PFHxS in eggs from commercial laying hens and backyard chickens in Italy.

	Lower Bound (LB)		**Upper Bound (UB)**		***P*-value LB**	***P*-value UB**
	Commercial	Backyard	Commercial	Backyard		
PFOS	0.009 ± 0.01	0.659 ± 0.10	0.107 ± 0.01	0.732 ± 0.09	<0.001	<0.001
PFOA	0.000 ± 0.00	0.008 ± 0.01	0.114 ± 0.01	0.135 ± 0.01	0.45	0.10
PFNA	0.000 ± 0.00	0.032 ± 0.02	0.107 ± 0.01	0.175 ± 0.02	0.13	<0.001
PFHxS	0.009 ± 0.01	0.026 ± 0.01	0.117 ± 0.01	0.149 ± 0.01	0.43	0.04
Σ PFAS	0.018 ± 0.01	0.725 ± 0.11	0.444 ± 0.02	1.192 ± 0.11	<0.001	<0.001

The mean value of total PFAS reported in commercial eggs is 0.018 µg/kg versus 0.752 µg/kg for home produced eggs from backyard chickens in LB, and 0.444 µg/kg versus 1.192 µg/kg in UB. The most abundant contaminant in both categories is PFOS but while in commercial eggs it ranges from 0.009-0.107 µg/kg (LB-UB) in home produced eggs from backyard chickens ones it has much higher values, ranging between 0.659-0.732 µg/kg (LB-UB). PFNA values range from 0-000.107 µg/kg (LB-UB) for commercial eggs to 0.032-0.175 µg/kg (LB-UB) for home produced eggs from backyard chickens. Although the differences between PFOA and PFHxS were not statistically significant according to the p-value test, a slightly higher amount of these two analytes was reported in home produced eggs from backyard chickens, which have PFOA values between 0.008-0.135 µg/kg (LB-UB), while the commercial ones comprised between 0.000-0.114 µg/kg (LB-UB).

The number of quantifiable samples of eggs from backyard chickens was higher than those of commercial eggs for all analytes, but expecially for PFOS, which in home produced eggs from backyard chickens was detectable in 50% of the samples, compared to 2% for commercial eggs, as shown in figure 4.2.

Figure 4.2. Frequencies distribution of left censored (LC) data regarding the presence of different PFAS in eggs from commercial laying hens and backyard chickens in Italy.

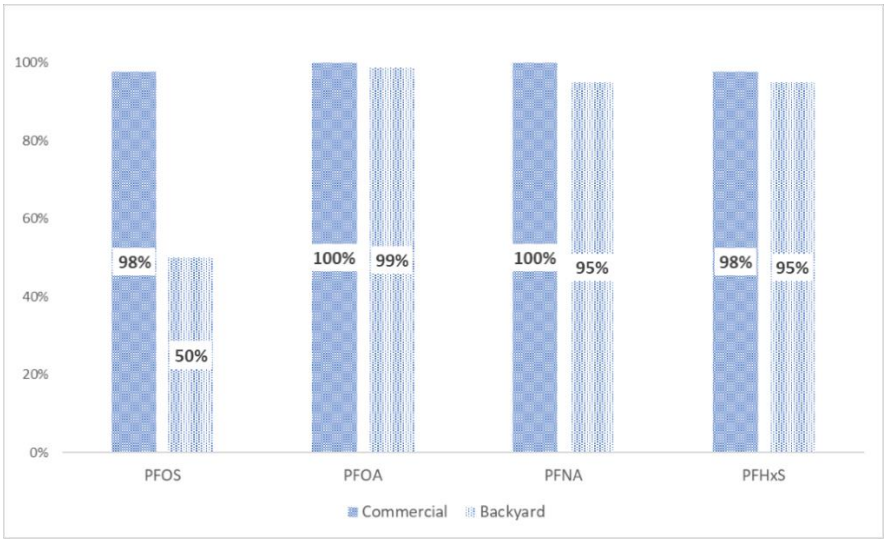
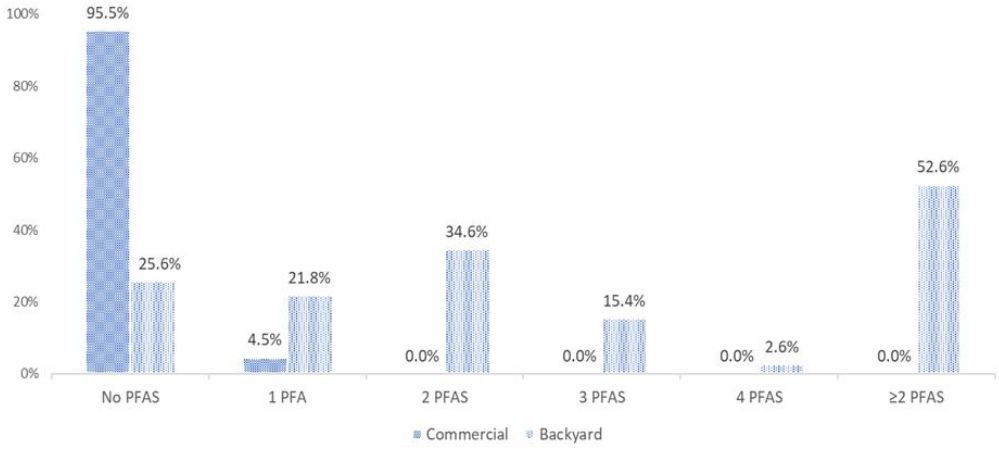


Figure 4.3 shows a different distribution of the co-presence of more than one PFAS in eggs produced with the two farming methods. Only the quantifiable samples were considered in the figure. As can be seen, in commercial eggs no more than two analytes have ever been quantified in the same sample, while in home produced eggs from backyard chickens 52.6% of the samples contained more than two PFAS at quantifiable levels.

Figure 4.3. Copresence of PFAS in eggs from commercial laying hens and backyard chickens in Italy.



Finally, is interesting to compare the data obtained with what is reported in the latest EFSA opinion (EFSA, 2020) regarding PFAS contamination in eggs, as shown in the table 4.8.

Table 4.8. Comparison of the mean levels in ppb (µg/kg) of PFOS, PFOA, PFNA and PFHxS in eggs measured in this thesis and the data reported for "eggs and egg products" in 2020 EFSA Opinion.

	PFOS			**PFOA**			**PFNA**			**PFHxS**		
	%LC	**LB**	**UB**	**%LC**	**LB**	**UB**	**%LC**	**LB**	**UB**	**%LC**	**LB**	**UB**
Home produced eggs	50%	0.659	0.732	99%	0.008	0.135	95%	0.032	0.175	95%	0.026	0.149
Commercial eggs	98%	0.009	0.107	100%	0.000	0.114	100%	0.000	0.107	98%	0.009	0.117
2020 EFSA Opinion	92%	0.270	0.350	92%	0.106	0.210	100%	0.000	0.098	97%	0.000	0.060

The percentage of quantifiable samples for PFOS was significantly higher in home produced eggs than the EFSA opinion, in which instead only 92% of the samples were quantifiable.

Also PFAS levels was higher, because that reported by EFSA are equal to 0,270-0,350 µg/kg (LB-UB), despite higher than that reported in commercial (0,009-0,107 µg/kg LB-UB). The detectable levels of the remaining analytes, on the other hand, are approximately the same as those reported by EFSA opinion, but while for PFOA the levels detected by EFSA (0.106-0.210 µg/kg LB-UB) are higher than those reported in this thesis in home produced and commercial eggs (0.008-0.135 µg/kg LB-UB and 0.000-0.114 µg/kg LB-UP respectively), PFNA and PFHxS have higher values in both home produced and commercial eggs, compared to the values reported by EFSA equal to 000.0 -0.098 µg/kg (LB-UB) and 0.000-0.060 µg/kg (LB-UB) respectively.

4.2 DRCs IN FOODS OF ANIMAL ORIGINS PURCHASED FROM ITALIAN AND FRENCH LARGE-SCALE RETAILERS

From the analyzes conducted in Italian and French procedural blank samples (BLK) it emerged that the BLK are devoid of DRC, with the exception of the DPs. DPs levels are quite low for Italian BLK and much higher for French ones. This made correct quantification difficult. We hypothesized that contamination came from the PLE instrument. For DPs, the mean procedural blank was deduced for IT samples on one side and for FR samples on the other, with respective standard deviation (SD). For the quantification in the food, only the samples that had an amount of DPs above the mean of the blanks plus the standard deviation multiplied by three times (M+3*SD of blanks) were considered.

4.2.1 Results obtained in Italian food samples

As shown in table 4.9, the total amount in pg of each DRCs contained in the blank and food samples was determined. For both syn and anti-DP the mean amount in procedural blanks corresponded to 48 pg, with a standard deviation of 38 and 22, respectively. By applying the formula M+3*SD only food samples with more than 162 and 115 total pg detected were considered for syn-DP and for anti-DP, respectively. This means that out of 28 samples in which syn-DP levels were detected, only 6 were quantified, following this criterion, while for anti-DP only 11 out of 30. The quantified samples for syn-DP and anti-DP are highlighted in green in table 4.9.

Table 4.9. Quantity per sample (pg) of DRCs in blank and food sample from Italy. For food samples are indicated the wet weight (ww), the lipid weight (lw) and the percentage of lipids in the samples (%lw). The quantified samples for syn-DP and anti-DP are highlighted in green. "-"= non detected.

Sample	Dec-601	Dec-602	Dec-603	CP	Syn-DP	Anti-DP	ww	lw	%lw
Blk_IT_1	-	-	-	-	22	41			
Blk_IT_2	-	-	-	-	35	34			
Blk_IT_3	-	-	-	-	41	46			
Blk_IT_4	-	-	-	-	28	31			
Blk_IT_5	-	-	-	-	115	86			
		Mean per sample (pg)			48	48			
		RSD%			79%	47%			
		SD			38	22			
		M+3*SD			**162**	**115**			
IT_1	-	-	-	-	37	88	16	06	4%
IT_2	-	-	-	-	89	**1307**	16	0.6	4%
IT_3	-	-	12	-	138	**140**	14	0.9	7%
IT_4	-	-	-	-	**703**	**483**	12	1.0	8%
IT_5	-	-	-	-	144	**178**	12	1.3	10%
IT_6	-	-	-	-	41	70	12	1.6	14%
IT_7	-	-	-	-	46	**116**	14	1.3	9%
IT_8	-	-	-	-	24	60	16	0.4	2%
IT_9	-	-	-	-	**1922**	**2156**	16	1.0	6%
IT_10	-	-	-	-	**4908**	**4593**	17	1.6	9%
IT_11	-	-	-	-	**568**	**683**	16	1.7	10%
IT_12	-	-	-	-	121	**134**	17	1.6	9%
IT_13	-	-	-	-	26	36	10	1.8	17%
IT_14	-	-	1	-	109	88	15	0.7	5%
IT_15	-	14	-	-	5	15	13	1.2	9%
IT_16	-	-	-	-	27	36	25	0.1	0%
IT_17	-	-	-	-	50	46	16	0.2	1%
IT_18	-	-	-	-	**1153**	**1310**	15	0.3	2%
IT_19	-	-	-	-	**465**	**530**	16	0.5	3%
IT_20	-	-	-	-	49	54	32	0.7	2%
IT_21	-	-	-	-	80	58	38	0.1	0%
IT_22	-	-	-	-	12	21	7	2.9	42%
IT_23	-	-	-	-	14	27	13	1.8	14%
IT_24	-	-	-	-	8	25	6	2.4	42%
IT_25	-	-	-	-	3	9	1	1.0	100%
IT_26	-	-	-	-	0	15	1	1.0	100%
IT_27	-	-	-	-	1	8	1	1.0	100%
IT_28	-	-	-	-	-	3	1	1.0	100%
IT_29	-	-	1	-	-	6	1,3	1.0	75%
IT_30	-	-	-	-	8	37	1,8	1.0	57%

For each food sample, as listed in the last 3 columns of table 4.9, the wet weight (ww), the lipid weight (lw) and the percentage of lipids were determined. These data allowed to calculate the content of each DRCs expressed on pg/g ww (dividing the amount of pg present in each sample by the respective wet weight of the sample) and on pg/g lw (dividing the concentration present in each sample expressed in pg/g ww by the respective percentage of lipids present in the sample) in food samples, as shown in the table 4.10.

Table 4.10. Quantity of DRCs (pg/g ww- pg/g lw) in food samples from France. "n.e."= Not Evaluable, refers to samples in which procedural contamination was too high for a correct quantifications. "-"= non detected.

Sample		Dec-601	Dec-602	Dec-603	CP	Syn-DP	Anti-DP
IT_1	Beef	-	-	-	-	n.e	n.e
IT_2	Beef	-	-	-	-	n.e	81-1999
IT_3	Beef	-	-	0.9-13	-	n.e	7-97
IT_4	Pork	-	-	-	-	53-661	35-440
IT_5	Pork	-	-	-	-	n.e	10-103
IT_6	Pork	-	-	-	-	n.e	n.e
IT_7	Poultry	-	-	-	-	n.e	5-53
IT_8	Poultry	-	-	-	-	n.e	n.e
IT_9	Poultry	-	-	-	-	116-1893	130-2129
IT_10	Eggs	-	-	-	-	281-3076	263-2877
IT_11	Eggs	-	-	-	-	32-308	39-376
IT_12	Eggs	-	-	-	-	n.e	5-55
IT_13	Fish	-	-	-	-	n.e	n.e
IT_14	Fish	-	-	0.1-2	-	n.e	n.e
IT_15	Fish	-	1.1-11	-	-	n.e	n.e
IT_16	Fish	-	-	-	-	n.e	n.e
IT_17	Fish	-	-	-	-	n.e.	n.e
IT_18	Seafood	-	-	-	-	76-3945	87-4507
IT_19	Seafood	-	-	-	-	26-801	30-928
IT_20	Milk	-	-	-	-	n.e	n.e
IT_21	Milk	-	-	-	-	n.e	n.e
IT_22	Cheese	-	-	-	-	n.e	n.e
IT_23	Cheese	-	-	-	-	n.e	n.e
IT_24	Cheese	-	-	-	-	n.e	n.e
IT_25	Oil	-	-	-	-	-	-
IT_26	Oil	-	-	-	-	-	-
IT_27	Oil	-	-	-	-	-	-
IT_28	Oil	-	-	-	-	-	-
IT_29	Animal fat	-	-	0.6-0.8	-	-	-
IT_30	Vegetable fat	-	-	-	-	-	-

CP and Dec-601 were not detected in any of the Italian food samples.
Dec-602 was detected only in one fish meat sample, at a concentration of 0.1 pg/g ww (11 pg/g lw).
Dec-603 was also detected in a few samples, with concentrations that had a range similar to Dec-602 values. Concerned matrices were beef meat (0.9-13 pg/g ww- pg/g lw), fish meat (0.1-2 pg/g ww- pg/g lw) and animal fat (0.6-0.8 pg/g ww- pg/g lw).
Regarding DPs contamination, it was the most abundant and high, in fact it was detected in two beef samples, two pork samples, two poultry samples and in all three eggs and all two seafood samples at higher concentrations than other DRCs. The most abundant levels of syn-DP and anti-DP on the wet weight refer to samples of eggs (281 pg/g ww and 263 pg/g ww, respectively) and poultry (116 pg/g ww and 130 pg/g ww, respectively). The most abundant ones on the lipid weight basis were detected in a seafood sample (3945 pg/g lw and 4507 pg/g lw, respectively) and in an egg sample (3076 pg/g lw and 2877 pg/g lw, respectively). The other syn-DP values ranged from 26-76 pg/g ww to 308-1893 pg/g lw and the anti-DP values from 5-87 pg/g ww to 53-2129 pg/g lw. Milk, cheese, oil and vegetable fat samples were the only matrices in which no contamination of any DCRs was detected.

4.2.2 Results obtained in French food samples

As shown in Table 4.11, the total amount in pg of each DRC contained in the blank and food samples was determined. For syn-DP the mean amount in procedural blanks was 3632 pg (SD equal to 4830), while for syn-DP the mean value in blanks was 1284 (SD of 1499). By applying the formula M+3*SD only food samples with more than a total of 17091 pg detected were considered for syn-DP and for anti-DP only food samples with more than 5218 total pg detected. Such intense procedural contamination meant that out of 25 samples in which syn-DP levels were detected, not one was quantified following this criteria, while for anti-DP only 1 in 30. Quantified sample for anti-DP are highlighted in green in Table 4.11.

Table 4.11. Quantity per sample (pg) of DRCs in blanck and food sample from France. For food samples are indicated the wet weight (ww), the lipid weight (lw) and the percentage of lipids in the samples (%lw). The quantified samples for anti-DP are highlighted in green. "-"= not detected.

Sample	Dec-601	Dec-602	Dec-603	CP	Syn-DP	Anti-DP	ww	lw	%lw
Blk_FR_1	-	-	-	-	1371	544			
Blk_FR_2	-	-	-	-	1214	692			
Blk_FR_3	-	-	-	-	1066	418			
Blk_FR_4	-	-	-	-	10875	3481			
		Mean per sample (pg)			3632	1284			
		RSD%			124%	102%			
		SD			4830	1499			
		M+3EcT			**17.091**	**5.218**			
FR _1	-	-	13	-	1307	1187	15	0.4	3%
FR _2	-	-	1	-	314	251	16	0.7	4%
FR _3	-	-	161	-	2254	1818	13	1.2	9%
FR _4	-	-	-	-	1308	1.363	14	1.3	9%
FR _5	-	-	-	-	4068	2.578	15	0.4	3%
FR _6	-	-	1	-	2085	1053	9	2.4	26%
FR _7	-	-	-	-	563	365	16	1.3	8%
FR _8	-	-	-	-	191	358	16	0.3	2%
FR _9	-	-	-	-	242	252	15	11	7%
FR _10	-	-	-	-	1176	1181	17	1.4	8%
FR _11	-	-	-	-	208	194	17	1.7	10%
FR _12	-	-	9	-	196	145	16	1.6	10%
FR _13	-	16	12	-	184	209	10	2.1	21%
FR _14	-	33	-	-	91	139	20	0.3	1%
FR _15	-	24	-	-	369	985	17	1.1	7%
FR _16	-	-	-	-	586	1075	21	0.1	1%
FR _17	-	-	-	-	166	181	15	0.1	1%
FR _18	-	-	-	-	7097	**7757**	16	0.3	2%
FR _19	-	-	-	-	742	441	14	0.7	5%
FR _20	-	-	-	-	130	90	31	0.7	2%
FR _21	-	-	-	-	1612	1309	37	0.2	0%
FR _22	-	-	-	-	393	297	7	2.3	34%
FR _23	-	-	-	-	91	121	12	1.8	14%
FR _24	-	-	-	-	123	125	9	1.9	21%
FR _25	-	-	-	-	-		1	1.0	100%
FR _26	-	-	-	-	-	5	1	1.0	100%
FR _27	-	-	-	-	-	11	1	1.0	100%
FR _28	-			-		7	1	1.0	100%
FR _29	-		2	-		8	1.2	1.0	85%
FR _30	-			-	9	16	1.3	1.0	80%

For each food sample, as listed in the last 3 columns of table 4.11, the wet weight (ww), the lipid weight (lw) and the percentage of lipids were determined. These data allowed to calculate the content of each DRCs expressed on pg/g ww (dividing the amount of pg present in each sample by the respective wet weight of the sample) and on pg/g lw (dividing the concentration present in each sample expressed in pg/g ww by the respective percentage of lipids present in the sample) in food samples, as shown in the table 4.12.

Table 4.12. Quantity of DRCs (pg/g ww- pg/g lw) in food samples from France. "n.e."= Not Evaluable, refers to samples in which procedural contamination was too high for a correct quantifications. "-"= non detected.

Sample		Dec-601	Dec-602	Dec-603	CP	Syn-DP	Anti-DP
FR _1	Beef	-	-	**0.9-34**	-	n.e	n.e
FR _2	Beef	-	-	**0.1-1**	-	n.e	n.e
FR _3	Beef	-	-	**12-139**	-	n.e	n.e
FR _4	Pork	-	-	-	-	n.e	n.e
FR _5	Pork	-	-	-	-	n.e	n.e
FR _6	Pork	-	-	**0.1-0**	-	n.e	n.e.
FR _7	Poultry	-	-	-	-	n.e.	n.e.
FR _8	Poultry	-	-	-	-	n.e.	n.e.
FR _9	Poultry	-	-	-	-	n.e.	n.e.
FR _10	Eggs	-	-	-	-	n.e.	n.e.
FR _11	Eggs	-	-	-	-	n.e.	n.e.
FR _12	Eggs	-	-	**0.6-6**	-	n.e.	n.e.
FR _13	Fish	-	**1.6-8**	**1.2-6**	-	n.e.	n.e.
FR _14	Fish	-	**1.6-131**	-	-	n.e.	n.e.
FR _15	Fish	-	**1.4-22**	-	-	n.e.	n.e.
FR _16	Fish	-	-	-	-	n.e.	n.e.
FR _17	Fish	-	-	-	-	n.e.	n.e.
FR _18	Seafood	-	-	-	-	n.e.	**410-25894**
FR _19	Seafood	-	-	-	-	n.e.	n.e.
FR _20	Milk	-	-	-	-	n.e.	n.e.
FR _21	Milk	-	-	-	-	n.e.	n.e.
FR _22	Cheese	-	-	-	-	n.e.	n.e.
FR _23	Cheese	-	-	-	-	n.e.	n.e.
FR _24	Cheese	-	-	-	-	n.e.	n.e.
FR _25	Oil	-	-	-	-	-	-
FR _26	Oil	-	-	-	-	-	-
FR _27	Oil	-	-	-	-	-	-
FR _28	Oil	-	-	-	-	-	-
FR _29	Animal fat	-	-	**1.9-2.3**	-	-	-
FR_30	Vegetable fat	-	-	-	-	-	-

Like for the Italian samples, also in the French ones CP and Dec-601 were not detected in any of the analyzed samples.

Dec-602 was detected in threes fish samples with values ranging from 1.4 to 1.6 pg/g on wet weight, and from 8 to 131 pg/g on lipid weight. These concentrations are in agreement with the level found in the only Italian sample in which Dec-602 was found, which is also fish meat.

Dec-603 was quantified in levels that are similar to the Italian ones, and range from 0.1-1.9 pg/g ww at 1-34 pg/g lw, with the exception of one beef sample, which showed higher levels of 12-139 pg/g ww- pg/g lw The French samples that showed quantifiable levels of Dec-603 are more numerous than the Italian ones, and include four samples of meat (including three beef meat and one pork meat), one sample of eggs, one of fish and one of animal fat.

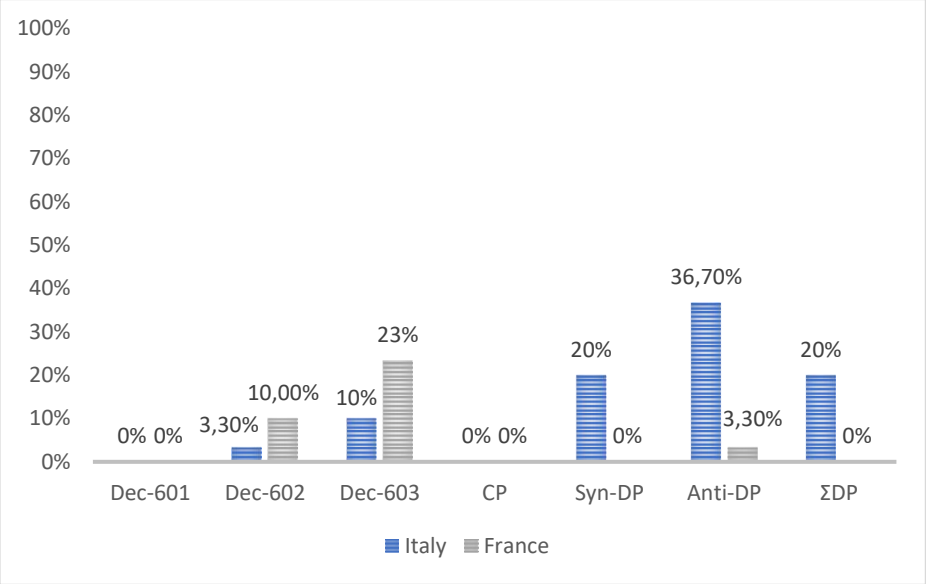
Regarding the determination of DPs, only one seafood sample and only for Anti-DP showed significantly higher levels than procedural contamination, quantified in 410-25894 pg/g ww - pg/g lw As for Italy, also French milk, cheese, oil and vegetable fat samples were the only matrices in which no contamination of any DCRs was detected, with poultry meat samples in addition.

4.2. Comparison between results in Italian and French samples

In figure 4.4. it is possible to see the differences between the percentage of Italian and French samples that showed quantifiable levels of the researched DRCs. For both countries, no sample contained traces of Dec-601 and CP.

Dec-602 and Dec-603 were detected both in Italy and in France, but while for Italy the percentage of positive samples was 3.3% for Dec-602 and 10% for Dec-603, in France it was more high, equal to 10% for Dec-602 and 23% for Dec-603. But the main difference concerns the percentage of positive samples for DPs: it would seem that in Italy the percentage is much higher, both for the Syn-DP (20% of the positive samples against 0% of the French samples) and for the Anti-DP (36.7% for Italy and 3.30% for France), but in reality this does not mean that the French samples were less contaminated. This difference is influenced by the high levels of procedural contamination found for the French samples, which drastically reduced the percentage of samples positive for DPs, making it difficult to compare the results correctly.

Figure 4.4. Comparison between the percentage of Italian and French samples quantified for each DRCs.



For the comparison of the results between the two countries, the samples were divided into five food categories, and for each one the mean value detected for each DRCs was calculated. The results obtained are expressed in table 4.13 for Italy and in table 4.14 for France.

Table 4.13 Average concentration of DRCs (pg/g ww- pg/g lw) contained in the food categories of the Italian samples.

	Dec-601	Dec-602	Dec-603	CP	Syn-DP	Anti-DP	ΣDP
Meat and meat products (n=9)	-	-	0.1-1.4	-	18.7-283.7	29.7-525.3	48.4-809
Eggs (n=3)	-	-	-	-	104.3-1128	102.3-1102.6	206.6-2230.6
Fish and seafood (n=7)	-	0.1-1.5	0.01-0.2	-	14.5-678	16.7-776.4	31.2-1454.4
Milk and dairy products (n=5)	-	-	-	-	-	-	-
Animal and vegetable fats and oil (n=6)	-	-	0.1-0.1	-	-	-	-

Table 4.14. Average concentration of DRCs (pg/g ww- pg/g lw) contained in the food categories of the French samples.

	Dec-601	Dec-602	Dec-603	CP	Syn-DP	Anti-DP	ΣDP
Meat and meat products (n=9)	-	-	1.4-19.3	-	-	-	-
Eggs (n=3)	-	-	0.2-2	-	-	-	-
Fish and seafood (n=7)	-	0.6-23	0.1-0.8	-	-	58.5-3699.1	58.5-3699.1
Milk and dairy products (n=5)	-	-	-	-	-	-	-
Animal and vegetable fats and oil (n=6)	-	-	0.3-0.3	-	-	-	-

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From the data expressed in tables 4.13 and 4.14 it emerges that the Italian samples of fish and seafood are the most contaminated by DRCs. Indeed, they contain Dec-602, Dec-602 and DPs. The French samples also confirm this trend. Indeed, although procedural contamination for DPs limited the number of samples with quantifiable levels, quantification of anti-DP in fish and seafood was possible due to the high levels, with an average of 58.5 pg/g ww and 3699.1 pg/g lw, slightly higher than the average of the Italian samples (16.7 pg/g ww and 776,4 pg/g lw). Dec-602 and Dec-603 instead, showed similar average levels for both Italy and France fish and seafood samples, equal to 0.1-1.5 pg/g ww- pg/g lw and 0.01-0.2 pg/g ww- pg/g lw for Italy and 0.6-23 pg/g ww- pg/g lw and 0.1-0.8 pg/g ww- pg/g lw for France.

In the Italian samples the second most contaminated category was meat and meat products, which showed quantifiable levels of both syn- and anti-DP (with an average of DPs content

equal to 48.4-809 pg/g ww- pg/g lw) and of Dec-603 (0.1-1.4 pg/g ww- pg/g lw) at slightly lower levels than those in the French samples (1.4-19.3 pg/g ww- pg/g lw), which however did not contain quantifiable levels of DP. The third most contaminated category in Italy was eggs, which contained only syn- and anti-DP, however at higher levels than all other food categories (104.3-1128 pg/g ww- pg/g lw for Syn-DP and 102.3-1102.6 pg/g ww- pg/g lw for Anti-DP). The French egg samples did not contain DPs, but unlike the Italian ones they showed quantifiable levels of Dec-603, equal to 0.2-2 pg/g ww- pg/g lw). Animal and vegetable fats and oil samples, both for Italy and for France, contained only Dec-603, at very low and similar levels (equal to 0.1-0.1 pg/g ww- pg/g lw and 0.3-0.3 pg/g ww- pg/g lw respectively). Finally, the least contaminated category for both French and Italian samples was milk and dairy products, where no sample showed quantifiable levels of any DRCs.

4.3 Comparison between the levels of DRCs reported in literature

To compare the data obtained in the Italian and French samples, a large bibliographic search of the currently published works on contamination by DRCs in food was carried out. Tables divided according to food category have been created, containing the average values of the DRCs reported in the works available in the literature. This extensive research and data processing was also previously published in a scientific journal (Gazzotti et al., 2021).

The studies in the literature that report the concentrations of DRCs in food for human consumption are currently scarce. Data available are much more numerous for fish than for all other food categories, because fish and aquatic organisms in general are often used to monitor pollution levels in aquatic ecosystems. They represent a critical link between the aquatic food network and population, in fact the consumption of contaminated fish is among the major source of exposure to environmental pollutants for the humans (Guo et al., 2019; Ren et al., 2013). This is also partially confirmed by the data obtained by analyzing the French and Italian samples, because for both the food category that presented the main contamination by DRCs was fish and seafood. In the collected literature, there are two other works that determine the DRCs content in fish samples collected in Italy (Giulivo et al., 2017) and in France (Abdel Malak et al., 2018), but both considered fish caught in local rivers, and therefore for environmental monitoring purposes, and not on commercial products as in the case of this study. The comparison of data is shown in Table 4.15 for fish

and sea products, Table 4.16 for meat and meat products, Table 4.17 for eggs, Table 4.18 for milk and dairy products and Table 4.19 for animal and vegetable fats. Furthermore, many works express concentration values in picogram per gram of wet weight (pg/g ww), while some report them in picogram per gram of lipid weight (pg/g lw); where possible both values are shown in the tables. These differences make comparison difficult given the various lipid content in foods. Furthermore, some authors use the lower bound (LB) and/or upper bound (UB) approach by analyzing data for left-censored values (results below the limit of detection-LOD or below the limit of quantification-LOQ). These data, if reported, have also been included in the tables, bearing in mind that the methods of calculating the LB and UB are not always standardized among the different works (Ghelli et al., 2021).

4.3.1 Fish and seafood products

Among all the DRCs, DP was the more frequently quantified compound in all the papers examined, with the only exceptions in the two works by Poma et al. (2016 and 2018) and in that of Tao et al. (2016). Also the Italian and French samples in this study follow this trend. As shown in Table 4.15, apart from the data obtained in the study by Wu et al. (2010) which are influenced by the fact that collected samples come from electronic waste treatment areas, the highest concentrations of ∑DP (223.21 x10^3 pg/g lw) were reported by Aznar Alemany et al. (2017) in commercial seafood available in European markets. Other high levels have been found in various studies and on freshwater fish in Europe (Giulivo et al., 2017; Abdel Malak et al., 2018; Santin et al., 2013; Sühring et al., 2013; Zacs et al., 2018) in Korea (Kang et al., 2010), and in various Korean seafood (especially shellfish), with values similar to those reported in our study in Italian (31.2-1454.4 pg/g ww- pg/g lw) and French samples (58.5-36991 pg/g ww- pg/g lw).

The data obtained by expressing the concentrations in ww obviously appear lower and also more homogeneous with each other, ranging from a few units to a few tens of ng/g.

Alongside the non-homogeneity of the data, there are various elements that make the comparison of data on fish and seafood complex. This could be attributed the phylogenetic variety of aquatic animals with consequent different positions in the food chain, metabolisms, lipid content, habitat (marine or freshwater) and production methods (fished or farmed). The widespread DP contamination in fish and seafood is an evidence of its global distribution both in the marine and freshwater environment.

The most frequently quantified DRC after DP is Dec-602, with the highest concentrations found in freshwater fishes in Spain (52.2 x10³ pg/g lw) (Santin et al., 2013). While this is not the case in the Italian and French samples: they showed the lowest levels of Dec-602 when compared to all other studies (0.1-1.5 pg/g ww- pg/g lw and 0.6- 23 pg/g ww- pg/g lw respectively) and much lower than the respective DP levels.

The highest mean value of Dec-603 (11.35 x10³ pg/g lw) was detected by Aznar Alemany et al. (2017) on fish and seafood from the European market. Values slightly higher than 2 x10³ pg/g lw were also found in freshwater fish from Spain. In almost all cases there is a simultaneous presence of Dec-602 and Dec-603 (often in smaller quantities). This is also confirmed by the Italian and French samples, in which was present also Dec-603, at levels equal to 0.01-0.1 pg/g ww-pg/g lw for Italy and 0.1-0.8 pg/g ww-pg/g for France.

Concerning CP and Dec-601, they were not found either in Italy or in France samples.

CP was quantified in four works. The highest concentration (100 pg/g lw LB) was detected in samples of catfish from France (Abdel Malak et al., 2018), followed by samples of salmon collected in Belgium (L'Homme et al., 2015) with a mean contamination level of 4.24 pg/g lw.

Contamination of Dec-601 was evaluated in only three works with, so far, undetected values (<1 pg/g ww).

Table 4.15 Comparison of data on DRCs concentration in fish and seafood reported in different studies (data are expressed as mean value in pg/g). "ND"=Not Detected"; "-"= not included in the study.

FISH AND SEAFOOD

Reference	Country	Unit	Scenario	Fish	*Dec-601*	*Dec-602*	*Dec-603*	*CP*	*syn-DP*	*anti-DP*	*ΣDP*
Abdel Malak et al., 2018	France	ww (lw)	LB-UB	Catfish (n=102)	ND	11.8-11.8 (555-555)	11.9-11.9 (499-501)	2.24-2.25 (100-102)	2.60-4.60 (189-506)	5.45-7.04 (370-637)	8.05-11.64 (559-1143)
Abdel Malak et al., 2019	Lebanon	ww	LB-UB	Fish (n=21)	0.0-0.3	7.0-7.8	0.2-0.3	0.4-0.5	2.0-4.2	3.0-3.9	5.0-8.0
Aznar Alemany et al., 2017	Europe	lw	UB	Fish and seafood (n=42)	-	70	11.35 x10³	-	63.78 x10³	159.43x10³	223.21x10³
Giulivo et al., 2017	Greece	lw	UB	Freshwater fish (n=4)	-	ND	ND	-	ND	ND	-
	Slovenia Croatia Bosnia-Herzegovina Serbia			Freshwater fish (n=10)	-	ND	ND	-	510	770	1.28 x10³
	Italy			Freshwater fish (n=13)	-	2.60 x10³	ND	-	ND	ND	-
Houde et al., 2014	Canada	lw	-	Yellow perch (n=29)	-	ND	ND	ND	ND	ND	ND
Kakimoto et al., 2012	Japan	ww	LB	Saltwater fish (n=20)	-	-	-	-	0.83	1.39	2.22
Kakimoto et al., 2014	Japan	ww	-	Fish, shellfish, their products (n=17)	-	-	-	-	1.0	0.9	1.9
Kang et al., 2010	Korea	lw	-	Freshwater fish (n=22)	-	-	-	-	8.1 x10³	16.9 x10³	25.0 x10³
Kim et al., 2014	Korea	ww (lw)	LB	Fish and shellfish (n=70)	-	3.99 (166.04)	ND (0.55)	-	8.25 (316.33)	28.09 (1031.95)	36.34 (1348.28)
Klosterhaus et al., 2012	USA	lw		Fish (n=14)	-	-	-	-	ND	957	957

L'Homme et al., 2015	Belgium	lw	UB	Salmon (n=8)	-	1.75	3.72	4.24	4.24	1.89	6.13
Poma et al., 2016	Belgium	ww	LB	Fish and fish products (n= 11)	-	-	-	-	ND	ND	ND
Poma et al., 2018	Belgium	ww	LB	Fish and fish products (n= 61)	-	-	-	-	ND	ND	ND
Ren et al., 2013	China	lw	-	River fish (n=149)	-	-	-	-	82	141	223
Rjabova et al., 2016	Latvia	lw	-	Baltic salmon (n=25)	-	370.0	36.4	-	85.6	159.0	244.6
Santín et al., 2013	Spain	lw	-	Freshwater fish (n=48)	-	52.2x10³	2.6 x 10³	-	520	620	1.14 x 10³
Sühring et al., 2013	Germany	ww (lw)	-	European eel (n=45)	-	600 (1.17x10³)	ND (10)	-	20 (590)	10 (180)	30 (770)
Sühring et al., 2016	Germany	ww	-	Freshwater fish (n=44)	-	77	ND	ND	20	3	23
Tao et al., 2016	Vietnam	lw	-	River fish (n=5)	-	-	-	-	ND	ND	ND
Tomy et al., 2007	Canada	lw		Freshwater fish (n=44)	-	-	-	-	183	259	442
Vaccher et al., 2020	Cameroon	ww	LB-UB	Fish (n=4)	0.00-0.37	6.34-6.64	0.21-0.64	0.51-0.52	0.89-1.59	1.00-1.31	1.89-2.90
	Mali			Fish (n=2)	0.00-0.80	27.55-27.55	1.32-1.33	1.15-1.16	5.68-7.87	10.19-11.26	15.87-19.13
	Benin			Fish (n=2)	0.00-1.03	4.37- 4.38	0.71- 0.88	0.69-0.71	3.68-5.55	5.88- 7.25	9.56- 12.80
	Nigeria			Fish (n=1)	0.00-0.10	11.07-11.07	0.00- 0.38	0.46-0.46	0.69-0.87	1.23- 1.31	1.92- 2.18
Wu et al., 2010	China	lw		Freshwater fish (n=86)	-	-	-	-	119.9 x10³	219.8 x 10³	339.7 x10³
				Control freshwater fish (n=5)	-	-	-	-	1.4 x10³	7.4 x10³	8.8 x10³
Zacs et al., 2018	Latvia	lw	LB	European eel (n=58)	-	250	10	-	60	200	260
Zacs et al., 2021	Latvia	ww	LB-UB	Fish (n=8)	-	15.94-16.16	18.24-18.78	-	5.01-5.01	9.45-9.45	14.46-14.46
This study	**Italy**	**ww-lw**	**LB**	**Fish and seafood (n=9)**	**ND**	**0.1-1.5**	**0.01-0.2**	**ND**	**14.5-678**	**16.7-776.4**	**31.2-1454.4**
This study	**France**	**ww-lw**	**LB**	**Fish and seafood (n=9)**	**ND**	**0.6-23**	**0.1-0.8**	**ND**	**ND**	**58.5-3699.1**	**58.5-3699.1**

4.3.2 Meat and meat products

Also in this case, among all the DCRs, DP was the most present contaminant in the considered works. Again, excluding data obtained in electronic waste treatment areas Vietnam (Tao et al., 2016) the highest mean concentrations of DP were measured in Korea (51.86 pg/g ww; 959.36 pg/g lw, LB scenario), with values very similar to those found in Italian samples (48.4-809 pg/g ww- pg/g lw). Lower values (with a range from 1.5 to 20.13 pg/g ww) were measured in samples collected in Lebanon, Japan, Belgium, sub-Saharan countries and Latvia. Other studies, conducted on a small number of samples, gave negative results (Tao et al., 2016; Poma et al., 2016), as well as the French samples.

Dec-602 and Dec-603 were the most often quantified alternative compounds to DP, but the values of these DRCs are generally low. The highest levels of Dec-603 are those of French samples (1.4-19.3 pg/g ww- pg/g lw). In the Italian samples and in the others study the levels are quite low and very similar, ranging from 0.1-0.59 pg/g ww LB. Dec-602 was not found in Italian and French samples, while in the other studies the level are very low, ranging from 0.0-1.8 pg/g ww LB, with the exception of Kim et al. (2014) that found levels equal to 3.54 -21.55 pg/g ww- pg/g lw LB. Concerning CP and Dec-601contamination, the levels detected in the studies were very low and negligible.

Table 4.16 Comparison of data on DRCs concentration in meat and meat products reported in different studies (data are expressed as mean value in pg/g). "ND"=Not Detected"; "-"= not included in the study.

MEAT AND MEAT PRODUCTS

Reference	Country	Unit	Scenario	Food	*Dec-601*	*Dec-602*	*Dec-603*	*CP*	*syn-DP*	*anti-DP*	*ΣDP*
Abdel Malak et al., 2019	Lebanon	ww	LB-UB	Meat and poultry (n=12)	0.0-0.2	0.0-0.7	0.1-0.3	0.0-0.05	10.1-1.8	7.4-8.1	17.4-19.9
Kakimoto et al., 2014	Japan	ww	-	Meat and eggs (n=13)	-	-	-	-	0.6	0.9	1.5
Kim et al., 2014	Korea	ww (lw)	LB	Meat and meat products (n=35)	-	3.54 (21.55)	ND	-	11.61 (234.74)	40.25 (724.62)	51.86 (959.36)
L'Homme et al., 2015	Belgium	lw	UB	Meat and poultry (n=16)	-	0.43	0.20	0.08	7.14	2.88	10.02
Poma et al., 2016	Belgium	ww	LB	Meat and meat products (n=1)	-	-	-	-	ND	ND	ND
Poma et al., 2018	Belgium	ww	LB	Meat and poultry (n=3)	-	-	-	-	2	8	10
Tao et al., 2016	Vietnam	lw	-	Chicken (n=15)	-	-	-	-	693 x 10^3	1683 x 10^3	2376 x 10^3
	Vietnam	lw	-	Pork (n=2)	-	-	-	-	ND	ND	ND
	Vietnam	lw	-	Control chicken (n=4)	-	-	-	-	ND	ND	ND
	Vietnam	lw	-	Control pork (n=1)	-	-	-	-	ND	ND	ND
Vaccher et al., 2020	Cameroon	ww	LB-UB	Meat (n= 2)	0.00-0.20	0.80- 0.93	0.00-0.75	0.11- 0.12	6.09-6.73	14.04-14.72	20.13- 21.45
	Mali			Meat (n= 1)	0.00-0.12	1.80- 1.80	0.00-0.42	0.00-0.01	1.66-3.16	2.10-2.83	3.76-5.99
	Benin			Meat (n= 2)	0.00-0.15	1.56- 1.56	0.00-0.24	0.00-0.02	0.57-1.19	1.61-2.07	2.18- 3.26-
	Nigeria			Meat (n= 2)	0.00-0.25	1.41- 1.41	0.00-0.88	0.00-0.04	2.02-2.26	4.29-4.39	6.31-6.65
Zacs et al., 2021	Latvia	ww	LB-UB	Meat (n= 8)	-	0.67-1.38	0.59-1.43	-	2.64-2.64	5.88-5.88	8.52-8.52
This study	**Italy**	**ww-lw**	**LB**	**Meat and meat products (n=9)**	**ND**	**ND**	**0.1-1.4**	**ND**	**18.7-283.7**	**29.7-525.3**	**48.4-809**
This study	**France**	**ww-lw**	**LB**	**Meat and meat product (n=9)**	**ND**	**ND**	**1.4-19.3**	**ND**	**ND**	**ND**	**ND**

4.3.3 Eggs

As for fish and meat, among all the DCRs, the DP was the most present contaminant in the considered works, and the only one quantified in our study in Italian eggs.

Excluding data obtained in samples collected near electronic waste treatment areas, the highest mean concentration of DP has been observed in Chinese chicken eggs with a value of 123.6 x 10^3 pg/g lw (Zhenh et al., 2012). These egg samples had been collected in southern China and used as a reference value to compare with that of contaminated areas. The second highest value in egg and egg products is one reported in Italian samples, with a mean value of 206.6-2230.6 pg/g ww- pg/g lw similar to those reported in Belgium by Poma et al. (2018) (159 pg/g ww), while the French samples did not show quantifiable levels of DP. Lower values (with a range from 1.27 to 30.33 pg/g ww) were measured in samples from Lebanon, Japan, Korea, Belgium, sub-Saharan countries and Latvia. It is well known that eggs can be a good environmental indicator of persistent organic contamination (Kim et al., 2014). This is why some studies have focused on DP contamination in wild bird eggs. In the latter, the levels measured are usually higher than those measured for chicken eggs. The reason can be the fact that in wild birds the body burden is high due to slower depuration because fewer eggs laid (Kim et al., 2014), possibly higher longevity and/or higher trophic level.

The highest values of Dec-602 (1.2-1.7 pg/g ww LB UB) and CP (1.36-1.41 pg/g ww LB UB) are those found in the study conducted in Lebanon (Abdel Malak et al., 2019). For Dec-601 instead, no values higher than the LOQ were obtained. The Italian and French samples showed no quantifiable levels of any of these 3 contaminants, while the French ones showed quantifiable levels of Dec-603 (0.2-2 pg/g ww- pg/g lw), with values similar to those reported by L'Homme et al. (2015) (2.76 pg/g lw), but lower than those reported by Vaccher et al. (2020) in Cameroon 1.8 pg/g ww.

Table 4.17 Comparison of data on DRCs concentration in eggs and egg products reported in different studies (data are expressed as mean value in pg/g). "ND"=Not Detected"; "-"= not included in the study.

EGGS

Reference	Country	Unit	Scenario	Food	*Dec-601*	*Dec-602*	*Dec-603*	*CP*	*syn-DP*	*anti-DP*	*ΣDP*
Abdel Malak et al., 2019	Lebanon	ww	LB-UB	Egg (n=5)	0.0-0.3	1.2-1.7	0.2-0.4	1.36-1.41	1.7-3.1	5.2-5.8	6.9-8.9
Kakimoto et al., 2014	Japan	ww	-	Meat and eggs (n=13)	-	-	-	-	0.6	0.9	1.5
Kim et al., 2014	Korea	ww (lw)	LB	Egg (n=5)	-	ND	ND	-	3.19 (17.64)	12.12 (67.32)	15.31 (84.96)
L'Homme et al., 2015	Belgium	lw	UB	Egg (n=8)	-	1.28	2.76	0.94	20.00	6.27	26.27
Poma et al., 2016	Belgium	ww	LB	Egg (n=2)	-	-	-	-	ND	ND	ND
Poma et al., 2018	Belgium	ww	LB	Egg and egg products (n=4)	-	-	-	-	32	127	159
Tao et al., 2016	Vietnam	lw	-	Chicken egg (n=15)	-	-	-	-	140 x 10^3	450 x 10^3	590 x 10^3
	Vietnam Japan	lw	-	Control chicken egg (n=2)	-	-	-	-	ND	ND	ND
Vaccher et al., 2020	Cameroon	ww	LB-UB	Eggs (n= 1)	0.00- 0.06	0.57- 0.65	1.80- 1.80	0.00- 0.01	2.02- 2.45	6.46- 6.64	8.48- 9.09
	Mali			Eggs (n= 1)	0.00- 0.26	0.79- 0.79	0.00- 0.31	0.00-0.05	0.00- 0.93	1.27- 1.74	1.27- 2.67
	Benin			Eggs (n= 1)	0.00- 0.06	0.00- 0.01	0.29- 0.29	0.00- 0.02	1.28- 1.67	3.50- 3.78	4.78- 5.45
	Nigeria			Eggs (n= 1)	0.00- 0.38	1.15- 1.15	0.00- 0.50	0.00- 0.08	1.82- 2.00	4.24- 4.32	6.06- 6.32
Zacs et al., 2021	Latvia	ww	LB-UB	Eggs (n= 8)	-	0.71-0.59	0.39-0.60	-	8.03-8.03	22.31-22.31	30.33-30.33
Zheng et al., 2012	China	lw		Chicken egg (n=33)	-	-	-	-	407 x 10^3	1192 x 10^3	1599 x 10^3
				Control Chicken egg (n=8)	-	-	-	-	28 x 10^3	95.6 x 10^3	123.6 x 10^3
This study	**Italy**	**ww-lw**	**LB**	**Eggs (n=3)**	**ND**	**ND**	**ND**	**ND**	**104.3-1128**	**102.3-1102.6**	**206.6-2230.6**
This study	**France**	**ww-lw**	**LB**	**Eggs (n=3)**	**ND**	**ND**	**0,2-2**	**ND**	**ND**	**ND**	**ND**

4.3.4 Milk and dairy products

No DRCs were detected in the Italian and French milk and dairy products samples.

In the others studies, among all the DCRs, the DP was the most present contaminant, excluding the work of Abdel Malak et al. (2019) where the main contaminant was Dec-602. The highest average concentrations of DP have been measured in Korea (23.87 pg/g ww- 928.52 pg/g lw). Lower values of DP were found in Lebanon (Abdel Malak et al. 2019), in Belgium (L'Homme et al., 2015; Poma et al., 2015), in the sub-Saharan countries (Vaccher et al., 2020), while it was not quantified in Japan (Kakimoto et al., 204) and in one sample from Belgium (Poma et al., 2016).

For this category, five papers among those consulted take into consideration the presence of other DRCs in addition to the DP.

The highest levels of Dec-602 contamination with similar mean values of about 3 pg/g ww, were measured in Lebanon (Abdel Malak et al., 2019) and Latvia (Zacs et al., 2021).

Dec-603 mean values are also higher in the study conducted in Latvia (2.39-2.80 pg/g ww LB-UB) (Zacs et al., 2021). CP contamination in milk and dairy products is very low.

Table 4.18 Comparison of data on DRCs concentration in milk and dairy products reported in different studies (data are expressed as mean value in pg/g). "ND"=Not Detected"; "-"= not included in the study.

MILK AND DAIRY PRODUCTS

Reference	Country	Unit	Scenario	Food	*Dec-601*	*Dec-602*	*Dec-603*	*CP*	*syn-DP*	*anti-DP*	*ΣDP*
Abdel Malak et al., 2019	Lebanon	ww	LB-UB	Milk and dairy products (n=13)	0.0-0.1	2.9-3.3	0.05-0.1	0.01-0.03	1.0-2.0	0.7-1.1	1.7-3.1
Kakimoto et al., 2014	Japan	ww	-	Milk and dairy products (n=5)	-	-	-	-	ND	ND	ND
Kim et al., 2014	Korea	ww (lw)	LB	Milk and dairy products (n=15)	-	ND	ND	-	4.42 (173.78)	19.45 (754.74)	23.87 (928.52)
L'Homme et al., 2015	Belgium	lw	UB	Milk (n=16)	-	0.89	1.06	0.26	12.50	5.11	17.61
Poma et al., 2016	Belgium	ww	LB	Milk (n=1)	-	-	-	-	ND	ND	ND
Poma et al., 2018	Belgium	ww	LB	Milk and dairy products (n=38)	-	-	-	-	2	7	9
Vaccher et al., 2020	Cameroon	ww	LB-UB	Milk and dairy products (n=1)	0.00-0.84	0.28-0.57	0.00-1.24	0.00-0.09	0.00-1.30	0.25-0.89	0.25-2.19
	Mali			Milk and dairy products (n=2)	0.00-0.18	0.31-0.31	0.00-0.16	0.02-0.04	0.21-0.98	0.39-0.90	0.60-1.88
	Benin			Milk and dairy products (n=3)	0.00-0.39	0.17- 0.17	0.00-0.31	0.00-0.02	0.07-0.75	0.06-0.69	0.13-1.44
	Nigeria			Milk and dairy products (n=1)	0.00-0.94	0.66- 0.66	0.00-0.66	0.00-0.13	0.66-1.29	2.18-2.46	2.84-3.75
Zacs et al., 2021	Latvia	ww	LB-UB	Milk and dairy products (n=8)	-	3.44-3.60	2.39-2.80	-	5.60-5.60	10.81-10.81	16.41-16.41
This study	**Italy**	**ww-lw**	**LB**	**Milk and dairy products (n=5)**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
This study	**France**	**ww-lw**	**LB**	**Milk and dairy products (n=5)**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**

4.3.5 Animal and vegetable fat

In vegetable oils and animal fat, DP has been quantified only in one out of four African states (Nigeria, Vaccher et al., 2020), Latvia (Zacs et al., 2021), Belgium (L'Homme et al., 2015) and Korea (Kim et al., 2014). The mean concentrations of DP how a tighter range (1.53-21.1 pg/g ww LB and 11.13-52.8 pg/g ww UB). Instead, in Japan and Belgium (Poma et al., 2016, 2018) as well for Italian and French samples, DP has not been quantified.

Dec-602 was quantified in 5 out of 6 studies (but not in the Italian and French samples), and was the compound that gave the highest mean value among the other compounds in the African states (Vaccher et al., 2020; Abdel Malak et al., 2019), respectively 3.0-11.8 pg/g ww LB-UB in Lebanon and from 1.5 to 3.0 pg/g ww in sub-Saharan countries.

Also Dec-603 was found in almost all the works, including Italian and France samples (0.1-0.1 pg/g ww-pg/g lw and and 0.3-0.3 pg/g ww-pg/g lw, respectively), with average values all quite similar. In Italian and France samples CP was not detected, and in the other works the values were rather low; the highest levels were measured in Lebanon (3.2-3.7 pg/g ww LB UB).

Dec-601 was the least detected analyte, and with the lowest levels.

Table 4.19 Comparison of data on DRCs concentration in animal and vegetable fat reported in different studies (data are expressed as mean value in pg/g). "ND"="Not Detected"; "-"= not included in the study.

ANIMAL AND VEGETABLE FAT

Reference	Country	Unit	Scenario	Food	*Dec-601*	*Dec-602*	*Dec-603*	*CP*	*syn-DP*	*anti-DP*	*ΣDP*
Abdel Malak et al., 2019	Lebanon	ww	LB-UB	Vegetable oil (n=7)	0.0-4.6	3.0-11.8	2.3-3.9	3.2-3.7	2.4-25.0	18.7-27.9	21.1-52.8
Kakimoto et al., 2014	Japan	ww	-	Oils and fats (n=4)	-	-	-	-	ND	ND	ND
Kim et al., 2014	Korea	ww	LB	Soy oil (n=5)	-	ND	ND	-	3.19	12.12	15.31
L'Homme et al., 2015	Belgium	lw	UB	Animal fat (n=18)	-	0.75	0.57	0.16	12.50	6.60	19.10
				Vegetable oil (n=2)	-	0.75	0.50	0.20	12.50	6.61	19.11
Poma et al., 2016	Belgium	ww	LB	Vegetable fat (n=1)	-	-	-	-	ND	ND	ND
Poma et al., 2018	Belgium	ww	LB	Animal and Vegetable fat (n=9)	-	-	-	-	ND	ND	ND
Vaccher et al., 2020	Cameroon	ww	LB-UB	Oil and fat (n= 3)	0.00-2.08	1.66-3.00	0.00-6.03	0.67-0.84	0.00-4.53	5.2-6.03	5.2-10.65
	Mali			Oil and fat (n= 2)	0.00-3.62	1.56-1.56	0.00-2.56	0.00-0.54	0.00-3.21	1.97-7.46	1.97-10.67
	Benin			Oil and fat (n= 2)	0.00-5.89	1.88-1.88	4.5-6.60	0.00-0.19	0.00-2.95	1.53-8.18	1.53-11.13
	Nigeria			Oil and fat (n= 2)	0.00-2.99	2..44-2.44	0.00-1.99	0.21-0.58	4.21-5.80	12.23-12.94	16.44-18.74
Zacs et al., 2021	Latvia	ww	LB-UB	Vegetable oil (n=4)	-	0.31-2.02	0.00-5.76	-	2.00-4.40	5.57-10.00	7.57-14.40
This study	**Italy**	**ww-lw**	**LB**	**Animal and vegetable fats and oil (n=6)**	**ND**	**ND**	**0.1-0.1**	**ND**	**ND**	**ND**	**ND**
This study	**France**	**ww-lw**	**LB**	**Animal and vegetable fats and oil (n=6)**	**ND**	**ND**	**0.3-0.3**	**ND**	**ND**	**ND**	**ND**

5. CONCLUSIONS

Environmental pollution is one of the main causes of food contamination and the presence of non-biodegradable chemicals that are accumulated by humans through the food chain is particularly concerned. In recent years there has been a growing interest from the scientific community in a particular class of environmental pollutants, defined as "contaminants of emerging interest" which include a large group of chemicals that are released into the environment by human activities and are generally persistent, ubiquitous and bioaccumulative along the food chain, reaching humans and causing known or suspected harmful effects on human health, but which are currently not monitored. Two important classes of emerging contaminants are PFAS and DRCs, which are also considered by the Stockholm Convention on Persistent Organic Pollution.

Among the numerous publications on PFAS levels in foods, which underline that fish and seafood in general are the most contaminated food categories (EFSA, 2018), few have focused specifically on chicken eggs (D'Hollander et al., 2011; Zafeiraki et al., 2016), while most of the scientific works concern wild bird eggs (Miller et al., 2015; Letcher et al., 2015) or other wild animal species related to environmental biomonitoring studies. Chicken eggs are an important part of the human diet and their consumption did not have any kind of limitations (ethical, religious, economic and environmental), but information on their contamination by PFAS is still very scarce, especially in Italy.

On the others hand, concern about DRCs environmental and biota contamination is relatively recent since the first detection was only reported in 2006 by Hoh et al. (2006). Therefore, the specific researches dedicated to the presence of these contaminants in food are currently still very limited. The category of foods that is most often taken into consideration is also in this case fish and seafood, because these organisms are often used for monitoring purpose. The other food categories, on the other hand, are rarely studied.

The aim of this thesis was to investigate these two categories of emerging contaminants, determining the levels of PFAS in commercial and domestic Italian eggs, and DRCs in different food samples purchased from Italian and French large-scale retailers.

The results on eggs show relatively uniform PFAS contamination, for both domestic and commercial eggs, but at different levels. PFOS is the most abundant and widespread contaminant. The data obtained allow to highlight the difference compared to eggs from

commercial laying hens, which showed lower levels of contamination. This can be explained by the fact that PFASs are persistent environmental pollutants and therefore the hens that live outdoors and have the possibility to scratch in the soil, as in the case of backyard chickens, are more exposed to these substances compared to hens raised in industrial systems (Zafeiraki et al., 2016). We can concluded that, according to Brambilla et al. (2015) eggs from rural flocks may represent a PFAS source, in particular PFOS.

Regarding DRCs contamination in food samples from Italy and France, the analytical conditions not yet fully optimized for this type of analysis and the procedural contamination encountered in the instruments involved in the preparation of the samples led to an higher uncertainty of the real values of DP contamination (which are the main DRCs studied), especially in the French samples. Despite this, for both France and Italy, the food category most contaminated by DP and other DRCs in general was that of fish and seafood.
This trend, also highlighted in the considered published works, confirms that, as for PFAS and POPs in general, fish and fish products are one of the main sources of food exposure for humans to these contaminant (Domingo et al., 2007). Although the production of DRCs is limited to a few sites in the world, the data analysis shows that the contamination of the DRCs is a global reality, despite the comparison of the data highlights the significant impact of the e-waste treatment areas on the environmental spread of the DRCs.
The Italian samples, unlike the French, showed DP contamination also in meat and eggs, and in eggs at levels higher than all other food categories considered, and with values even higher than the average values reported in literature. From the comparison with the reported works, it emerged that eggs, after fish, have the highest average levels of DP.
This, added to the results obtained for PFAS on eggs, highlights how eggs are also an important source of food exposure to emerging contaminants.
The other DRCs (in particular Dec-602 and Dec-603) were detected in the same food categories in Italy and France, but at slightly higher levels in the French samples. In the works present in the literature, data on other DRCs are still very few.
The difficulty of comparing the data obtained is also given by the different methods of expression of the results that are reported in the other works in the literature: the results are expressed according to lipid weight and/or wet weight. This, due to lipophilicity of these contaminants and the different lipid content of the various foods, makes it difficult a correct comparison of the data. For these reasons it is desirable to produce more works and data that determine the level of contamination by acting on three main factors: target

compounds to be searched (it is necessary to extend the studies also to other DRCs, and not only to DPs), differentiation of the analyzed matrices (more data on other food categories, apart from fish, are needed) and finally geographical areas of origin of the matrices (in order to have a general and equally distributed picture of contamination worldwide).

In conclusion, the general trend also confirmed by the available literature, shows that one of the main sources of dietary exposure for humans to some classes of emerging contaminants are fish products, to which the category of eggs is also added, as has been shown a source of exposure not to be underestimated.

6. BIBLIOGRAPHY

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ABSTRACT

Environmental pollution is one of the main causes of food contamination and the presence of non-biodegradable chemicals that are accumulated by humans through the food chain is particularly dangerous. In recent years there has been a growing interest from the scientific community in a particular class of environmental pollutants, defined as "contaminants of emerging interest" which include a large group of chemicals that are released into the environment by human activities and are generally persistent, ubiquitous and bioaccumulative along the food chain, reaching humans and causing known or suspected harmful effects on human health, but which are currently not monitored. Two important classes of emerging contaminants are PFAS and DRCs.

Among the numerous publications on PFAS levels in foods, which underline that fish and seafood in general are the most contaminated food categories, few have focused specifically on chicken eggs, that represent an important part of the human diet and their consumption did not have any kind of limitations (ethical, religious, economic and environmental).

On the others hand, concern about DRCs environmental and biota contamination is relatively recent since the first detection was only reported in 2006.Therefore, the specific researches dedicated to the presence of these contaminants in food are currently still very limited.

The aim of this thesis was to investigate these two categories of emerging contaminants, determining the levels of PFAS in commercial and domestic Italian eggs, and DRCs in different food samples purchased from Italian and French large-scale retailers.

The results show relatively uniform PFAS contamination and a more widespread presence of PFOS, for both domestic and commercial eggs, but at much higher levels in domestic eggs. This can be explained by the fact that PFASs are persistent environmental pollutants and therefore the hens that live outdoors and have the possibility to scratch in the soil, as in the case of backyard chickens, are more exposed to these substances compared to hens raised in industrial systems.

Regarding DRCs contamination the analytical conditions not yet fully optimized for this type of analysis and the procedural contamination encountered in the instruments involved in the preparation of the samples led to an underestimation of the real values of DP contamination (which are the main DRCs studied), especially in the French samples. Despite this, for both France and Italy, the food category most contaminated by DP and other DRCs in general was that of fish and seafood.

This trend, also highlighted in the considered published works, confirms that, as for PFAS and POPs in general, fish and fish products are one of the main sources of food exposure for humans to these contaminant. The Italian samples, unlike the French, showed DP contamination also in meat and eggs, and in eggs at levels higher than all other food categories considered, and with values even higher than the average values reported in literature. From the comparison with the reported works, it emerged that eggs, after fish, have the highest average levels of DP. This, added to the results obtained for PFAS on eggs, highlights how eggs are also an important source of food exposure to emerging contaminants. In conclusion, the general trend also confirmed by the available literature, shows that one of the main sources of dietary exposure for humans to some classes of emerging contaminants are fish products, to which the category of eggs is also added, as has been shown a source of exposure not to be underestimate.